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Étude de l'émergence et de la dynamique évolutive d'*Armillaria ostoyae*, agent pathogène du pin maritime

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AVANT-PROPOS

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CHAPITRE I

Introduction générale

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I. Rôles et stratégies évolutives des champignons dans les écosystèmes

Le règne des champignons (Ascomycètes, Basidiomycètes, Chytridiomycètes, Glomeromycètes et Zygomycètes) est constitué d'organismes hétérotrophes incapables de synthétiser par eux-mêmes leurs composants nutritifs, qui participent à divers processus écologiques dans tous les écosystèmes, qu'ils soient terrestres ou marins (Hyde *et al.* 1998 ; Dighton *et al.* 2005). Parmi eux, les champignons saprophytes ont un rôle essentiel dans le fonctionnement des écosystèmes en recyclant la matière organique (végétale et animale). Selon Floudas *et al.* (2012), l'émergence et l'expansion de familles de gènes associées à la dégradation de la lignine chez de nombreux champignons saprophytes, auraient pu être à l'origine de la fin de l'important stockage du carbone dans le sol à la fin du Carbonifère. La diversité spécifique de ces champignons est très importante, notamment en raison des espèces colonisant spécifiquement le substrat d'un hôte particulier, et de certaines espèces qui colonisent uniquement la litière fraîche, la litière décomposée, ou encore la matière organique du sol (Lodge & Cantrell 1995). Ces espèces peuvent représenter à elles seules jusqu'à 40 % de la biomasse totale d'un écosystème (Boddy & Watkinson 1995). En plus de cette fonction de recyclage du carbone du sol, certaines de ces espèces peuvent occasionnellement parasiter des hôtes ligneux affaiblis par des stress biotiques (liés à des bio-agresseurs, comme des insectes par exemple) ou abiotiques (liés par exemple à des événements de sécheresse). Ce parasitisme est alors qualifié de parasitisme secondaire (ou d'équilibre), car il ne survient qu'à la suite d'un, ou plusieurs facteurs de prédisposition, c'est-à-dire ayant préalablement influencés la sensibilité de l'hôte vis-à-vis de l'agent pathogène. Ce type de parasitisme contribue à accélérer la succession écologique dans un écosystème forestier en éliminant plus rapidement les individus matures (Ostry & Laflamme 2008). Cependant, certaines espèces disposent aussi de la capacité d'infecter des hôtes non affaiblis, et sont alors décrites comme parasites primaires. Ces stratégies des espèces (saprophytes ou parasites) peuvent évoluer en fonction du contexte environnemental, en particulier face aux changements globaux actuels. Certaines interactions impliquant les espèces de champignons et les arbres peuvent ainsi basculer d'un état non ou faiblement pathogène, au développement d'épidémies dévastatrices pour les populations de ligneux, posant alors la question des facteurs favorisant le développement de ces maladies.

II. Emergence des maladies fongiques des plantes

Ces épidémies sont souvent liées à l'introduction d'agents pathogènes résultant de l'intensification des échanges internationaux de plantes exotiques (en volume et en rapidité). Selon Anderson *et al.* (2004), près de 40% des émergences des maladies fongiques des plantes résulteraient ainsi de ces événements d'introductions. L'introduction, au début du 20^{e} siècle en Amérique du nord, du chancre du châtaignier (*Cryphonectria parasitica*) originaire d'Asie, a, par exemple, provoqué la quasi-extinction de la strate arborée du châtaignier américain (*Castanea dentata* ; Anagnostakis 1987). Cependant, ces émergences peuvent aussi résulter du développement d'espèces indigènes dont l'équilibre, préalablement établi avec l'hôte, a basculé en faveur du pathogène (Anderson *et al.* 2004). Cela s'explique notamment par l'augmentation du nombre de facteurs favorisant ces champignons pathogènes (Anderson *et al.* 2004 ; Wargo & Harrington 1991). Les changements climatiques récents par exemple, en raison de l'augmentation des événements météorologiques extrêmes, tels que les anomalies de pluviométrie et les variations plus importantes des températures, peuvent contribuer à augmenter la fréquence des stress hydriques et thermiques des plantes dont pourraient profiter des champignons pathogènes opportunistes tel que *Dothistroma septosporum* (Rosenzweig *et al.* 2001 ; Woods *et al.* 2005). De plus, il est suggéré que cet accroissement des températures pourrait aussi favoriser le développement des champignons phytopathogènes (Coakley *et al.* 1999 Harvell *et al.* 2002 ; Anderson *et al.* 2004), notamment en augmentant leur survie lors de la période hivernale, qui peut habituellement provoquer jusqu'à 99% de mortalité chez certains pathogènes, tel que *Phytophthora cinnamomi* (Marçais *et al.* 1996 ; Burdon & Elmqvist 1996). Ces changements climatiques pourraient également contribués à l'accroissement de la vitesse de leur cycle biologique ainsi que favoriser la distribution et l'abondance de leurs insectes vecteurs (Coakley *et al.* 1999 ; Harvell *et al.* 2002 ; Anderson *et al.* 2004).

Les changements de pratiques exercés sur les plantes cultivées peuvent également contribuer à la mise en place d'un environnement plus propice aux pathogènes et à leurs dispersions notamment en réduisant la biodiversité végétale (Figure 1 ; Fowler & Mooney 1990 ; Jactel *et al.* 2008 ; Stukenbrock & McDonald 2008). L'homogénéité des populations hôtes dans les écosystèmes agricoles (forte densité, faible variabilité génétique entre individus, et conditions environnementales optimisées), favorise en effet à la fois la multiplication et la transmission de l'agent pathogène, ainsi que l'absence de prédateurs ou d'antagonistes. Les forêts de plantations, qui sont majoritairement des monocultures, ont ainsi vu leur surface augmenter d'environ 30% en seulement 15 ans, passant alors de 175 millions

d'hectares en 1990, à 225 millions d'hectares en 2005 (Jactel *et al.* 2008). L'accroissement de la monoculture, au détriment des forêts mélangées, favoriserait alors le développement des maladies des arbres forestiers et notamment des champignons pathogènes (Pautasso *et al.* 2005 ; Koricheva *et al.* 2006). Les études sur le fomès (*Heterobasidion annosum*), illustrent par exemple l'effet de ces plantations monoculturales, une incidence plus faible de l'agent pathogène étant généralement observée dans les peuplements où les conifères sont mélangés avec des feuillus (Korhonen *et al.* 1998). Face à la demande croissante en ressources alimentaires (Godfray *et al.* 2010) et en bois respectivement (FAO 2013), de nouvelles techniques agricoles et sylvicoles se développent et peuvent contribuer à la réduction de la diversité génétique des espèces plantées et favoriser le développement des agents pathogènes. En effet, du fait de la sélection pour des critères de rendement, seuls quelques génotypes sont couramment utilisés dans ces écosystèmes agricoles, ce qui facilite donc l'adaptation des agents pathogènes (Edwards 1996 ; Zhu *et al.* 2000 ; Frey & Pinon 2004). En 1970, aux Etats Unis d'Amérique, 85% de la totalité du maïs cultivé dans le pays provenaient d'une seule variété, qui s'est malheureusement avérée très sensible à l'helminthosporiose (*Helminthosporium maydis*) et qui a diminué par 17% la production totale de maïs du pays (Tatum 1971; Ullstrup 1972). De plus, certaines pratiques sylvicoles et agricoles (labourage, irrigation et fertilisation des sols), homogénéisent également l'environnement sur d'importantes surfaces et minimisent donc les variations du sol qui pourraient pourtant intervenir dans la réduction des tailles de population de pathogène du sol. De surcroît, les techniques classiques utilisées en culture intensive des plantes imposent également aux pathogènes un type de sélection nettement différent de celui-ci imposé par les milieux naturels plus hétérogènes. En effet, les pathogènes des plantes cultivées doivent principalement faire face aux méthodes visant à limiter leur effet et reposant généralement sur l'utilisation de pesticides et de gènes de résistance. Cependant, lorsque le pathogène s'est adapté aux fongicides et que les résistances sont contournées, celui-ci peut alors très rapidement se propager à l'ensemble de la culture ou de la parcelle. Enfin, sous l'hypothèse d'un compromis entre la dispersion des pathogènes et leur virulence (May & Anderson 1983), l'intensification des systèmes de cultures tendrait à favoriser les parasites les plus virulents (au sens ici du taux de dégâts infligé à l'hôte). En effet, l'homogénéité des hôtes et leur forte densité permet aux pathogènes virulents de se propager rapidement d'hôte en hôte sans risque d'extinction par l'élimination trop rapide de la population hôte. Ainsi, une augmentation de l'intensité de virulence des pathogènes a pu être observée dans des écosystèmes artificiels en comparaison d'écosystèmes naturels (Thrall & Burdon 1999). L'augmentation des plantations forestières monospécifiques dans le monde pourrait donc favoriser ce type d'évolution même si ces plantations sont pour la plupart encore assez récentes (Wingfield *et al.* 2015). De plus,

un caractère supplémentaire des écosystèmes forestiers est la gestion pluriannuelle des peuplements. Ainsi certaines pratiques sylvicoles, telles que les élagages et les coupes de taillis par exemple, peuvent être responsables de stress importants rendant les arbres forestiers plus vulnérables aux infections par des champignons phytopathogènes opportunistes (Hood & Sandberg 1989 ; Hood *et al.* 2002). Ces stress peuvent également être à l'origine des blessures au collet des arbres ou bien sur leurs racines traçantes, générées par la circulation, de plus en plus fréquente dans le peuplement, des engins forestiers (Vasiliauskas 2001).

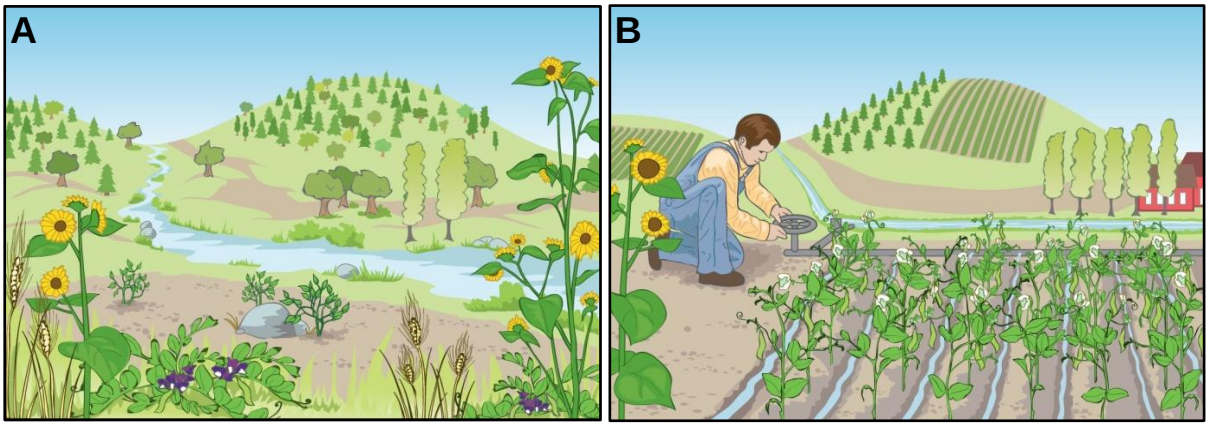


Figure 1 : Illustration des principales différences entre les écosystèmes naturels (**A**) et les écosystèmes agricoles (**B**) (Stukenbrock & McDonald 2008).

L'émergence de maladies fongiques en forêt résulte donc de phénomènes complexes mettant en jeu des caractéristiques propres au cycle biologique du pathogène, mais également les variations climatiques et les activités humaines, ainsi que la composition, la connexion et l'évolution du paysage forestier (Castello *et al.* 1995 ; Holdenrieder *et al.* 2004). Mais l'intensification des nouvelles pratiques sylvicoles contribuerait, semble-t-il, pour une grande part à l'accroissement des pertes dues aux maladies fongiques actuelles et futures (Delatour *et al.* 1985 ; Pautasso *et al.* 2005 ; Ennos 2015 ; Wingfield *et al.* 2015).

III. Contexte du massif forestier des Landes de Gascogne

1. L'histoire ancienne du massif forestier

Les surfaces de forêts dans les Landes de Gascogne ont fortement changé au cours du Quaternaire (-2,5 million d'années jusqu'à nos jours) (Frenzel *et al.* 1992 ; Jolivet *et al.* 2007). Par exemple, les changements climatiques majeurs de la dernière ère glaciaire (-110000 à -10000 ans) ont fortement changé la couverture et la composition des forêts

d'Europe. Le sud-ouest de la France n'a pas fait exception, et était alors essentiellement recouvert par la toundra ainsi que de quelques îlots forestiers composés d'une végétation boréale dans les régions les plus au sud ; cette réduction ayant probablement atteint son apogée lors du maximum glaciaire de cette dernière glaciation (-20000 à -18000 ans). Durant le postglaciaire et jusqu'au Suboréal (-10000 à -2700 ans), l'augmentation progressive des températures contribue à la recolonisation de la région par les forêts depuis les refuges climatiques d'Espagne (Taberlet *et al.* 1998). Les premières mentions du pin maritime lors de cette période remontent au Boréal (-8800 à -7500 ans) selon l'étude des pollens (Paquereau 1964). Cependant, l'accroissement des activités humaines lors du Subatlantique (-2700 ans jusqu'à nos jours) a une nouvelle fois engendré une réduction des forêts des Landes que l'on retrouvait majoritairement le long des rivières et des dunes Atlantiques (Vallauri *et al.* 2012). Ces surfaces forestières sont restées faibles jusqu'aux récentes plantations du 19^{e} siècle (Figure 2).

Figure 2 : frise chronologique représentant schématiquement l'évolution de la surface des forêts dans les Landes de Gascogne.

2. L'histoire récente du massif forestier

a. Les Landes avant la forêt de plantations

Le massif forestier des Landes de Gascogne est actuellement la plus importante forêt plantée d'Europe et l'une des principales sources de revenus financiers de la région Aquitaine avec plus de 3,5 milliards d'euros de chiffre d'affaires sur l'ensemble de la filière et représentant plus de 34000 emplois directs (www.aquitaine.fr). Bien que la forêt de pins des Landes constitue aujourd'hui, avec la vigne, l'un des paysages typiques de la région Aquitaine et qu'elle contribue également à forger l'identité landaise, ce massif forestier n'a été implanté dans ce territoire que très récemment. En effet, ce vaste territoire de près de 1,300 million d'hectares, était jusqu'en 1857 en grande partie initialement composé de landes et de marais, où les habitants vivaient essentiellement d'un système agro-pastoral (Figure 3A). Ce système reposait alors sur l'élevage ovin. Les moutons se nourrissaient sur les landes et leurs litières étaient utilisées comme fertilisants, permettant de cultiver ces sols d'une extrême

pauvreté en seigle, millet et panis. La forêt primitive, essentiellement composée de pin maritime et de chêne pédonculé, était majoritairement localisée dans les zones les plus drainées qui se trouvaient le long des cours d'eau et sur les dunes (Vallauri *et al.* 2012). Ces zones boisées correspondaient donc aux zones les plus hospitalières pour les habitants. La forêt naturelle entrait alors en concurrence directe avec les cultivateurs qui la défrichaient afin d'aménager leurs habitations, leurs champs et les zones de pâturage des moutons. Toutefois, dans les régions les plus boisées, le système agro-pastoral pouvait être complété par la culture du pin pour sa résine ("gemme"), et parfois, son bois en tant que combustible (Figure 3B).

Figure 3 : **A** : Photographie illustrant le système agro-pastoral des Landes de Gascogne. "Bergers à la Mouleyre", Commensacq, 1890 ; **B** : "Pins Lilère", Lüe, 1892 (F. Arnaudin, collection Musée d'Aquitaine, Bordeaux).

b. Les aménagements du territoire

Les premiers travaux d'aménagements du paysage, afin de stopper l'avancée des dunes littorales menaçant les villages côtiers, ont été entrepris par les Landais eux-mêmes et étaient principalement basés sur l'utilisation de semis de pins. Bien que le pin maritime ait toujours été présent dans la région, Guillaume Desbiey (1725-1785) et son frère Louis-Mathieu (1734-1802) sont les premiers à préconiser le semis de cette essence afin de valoriser le territoire ainsi que de stabiliser les dunes. Toutefois, l'ingénieur des Ponts et Chaussées Nicolas Brémontier (1738-1809), est l'un des acteurs le plus emblématique de l'installation des plantations du pin maritime dans la région. Ses travaux, reposant en grande partie sur ceux des frères Desbiey, ont permis de généraliser la technique d'ensemencement du pin maritime à l'ensemble du littoral atlantique des Landes. Par la suite, Jules Chambrelent (1817-1893), également ingénieur aux Ponts et Chaussées, est à l'origine des travaux expérimentaux d'assainissement du plateau landais. Ces expérimentations en grandeur nature, basées sur l'utilisation de plantations de pins maritimes et l'élaboration d'un système de drainage

original à partir de fossés, ont convaincu l'empereur Napoléon III lors de l'exposition universelle de 1855. La loi du 19 juin 1857 qui en découla, obligea alors les communes à assainir et à ensemencer la totalité de leurs landes communales, ou de les vendre si elles n'avaient pas les ressources pour financer ces travaux. Les 30 000 hectares de jeunes forêts incendiées qui suivirent (1868-1869), témoignèrent de l'opinion négative des Landais sur ces plantations, l'essentiel de leur mode de vie dépendant directement de la lande. Toutefois, avec l'augmentation du prix de la résine entre 1862 et 1865, les landais ont progressivement réorienté leur activité vers le gemmage. Une rapide transformation paysagère de la région c'est alors mise en place avec le remplacement de la majorité des landes par près d'un million d'hectares de forêt de pin maritime en seulement un demi-siècle (Figure 4 ; Thiveaud 1992 ; Dupuy 1994 ; Vallauri *et al.* 2012).

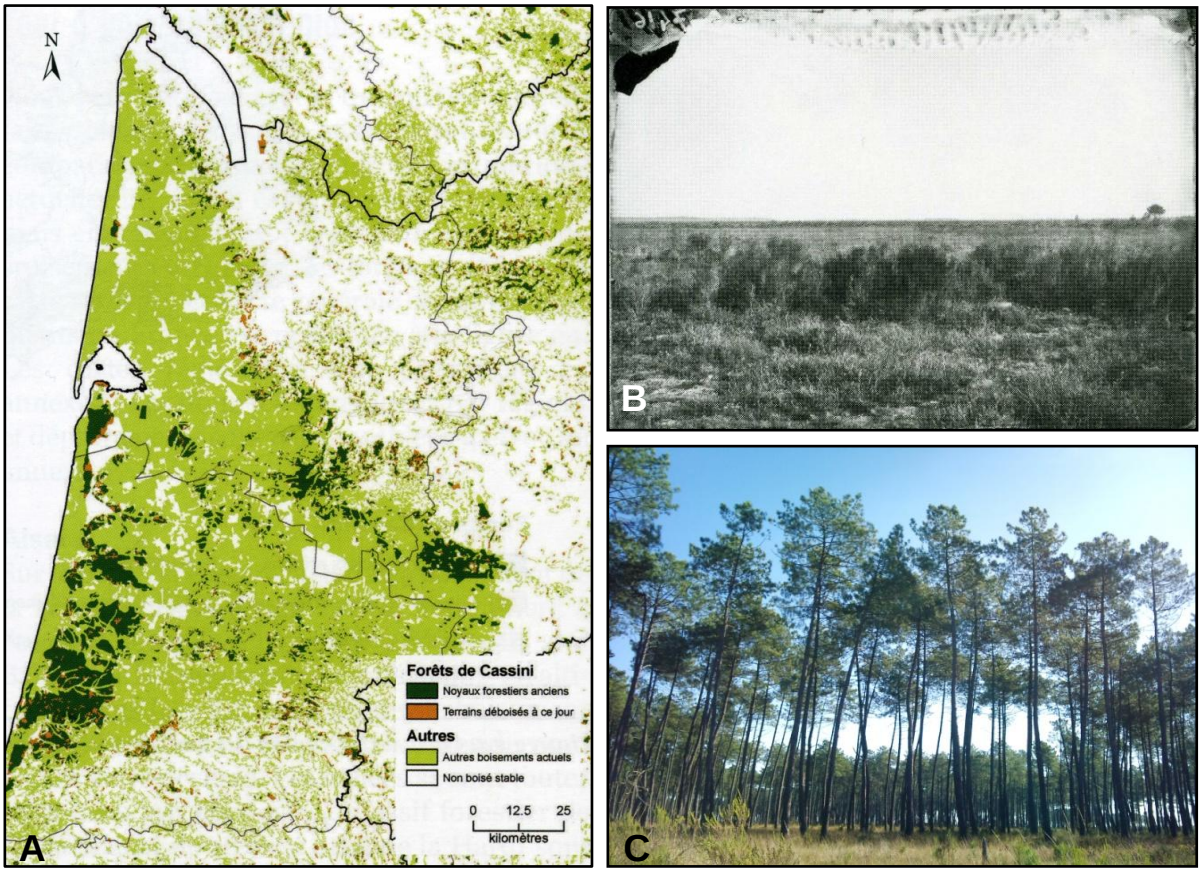


Figure 4 : **A** : Evolution de la couverture boisée en Aquitaine, à partir de la comparaison entre la carte de Cassini (1760-1788) et Corine Land Cover (2006) (Vallauri *et al.* 2012). **B** : Photographie illustrant les landes en 1876 (F. Arnaudin, "La Bruze et l'Aygue-Longue", collection Musée d'Aquitaine, Bordeaux). **C** : Photographie illustrant le massif forestier des Landes de Gascogne au 21^{e} siècle.

3. Evolution de la sylviculture dans le massif forestier

Les grands incendies de la Seconde Guerre mondiale (1937-1949), qui ont ravagé près de 40% de la surface totale du massif, ainsi que le développement de la pétrochimie, ont mis fin à la production de gemme des forêts landaises. A la suite de la mise en place de dispositif de lutte contre l'incendie et l'abandon du gemmage, la forêt des Landes de Gascogne s'est transformée en une forêt de production de bois dont les méthodes de gestion sylvicoles se sont progressivement intensifiées. La sylviculture moderne par l'assainissement, la multiplication des éclaircies, le raccourcissement des périodes de rotations entre les coupes rases, le débroussaillage, le travail mécanique du sol et la fertilisation phosphatée, a progressivement remplacé la méthode de gestion sylvicole ancestrale. Le remplacement petit à petit des bûcherons par des engins forestiers de plus en plus efficaces (abatteuses, porteurs…) a également contribué à l'augmentation de la mécanisation, depuis l'installation jusqu'à la récolte du peuplement de pins.

Actuellement, la méthode de gestion couramment employée dans le massif forestier repose sur une première éclaircie 10 à 15 ans après la plantation des jeunes pins maritimes (Figure 5). Par la suite, une deuxième, une troisième puis une quatrième éclaircie sont souvent réalisées après 15-20 ans, 20-25 ans et 25-35 ans respectivement. La coupe rase est généralement effectuée entre 35 et 55 ans à la suite de laquelle le reboisement est à nouveau initié entre 2 et 3 ans après la coupe rase. Cette sylviculture intensive semble propice au développement de l'Armillaire pour au moins deux raisons. Tout d'abord, les éclaircies entre 10 et 30 ans peuvent en effet lever les résistances des pins infectés par des nécroses latentes et servir de nouvel inoculum pour les arbres voisins. Ensuite, les parcelles sont constamment enrésinées et le temps de latence moyen de 2 ans entre la coupe rase et la replantation permettrait a priori le maintien de l'inoculum dans le sol entre deux plantations successives.

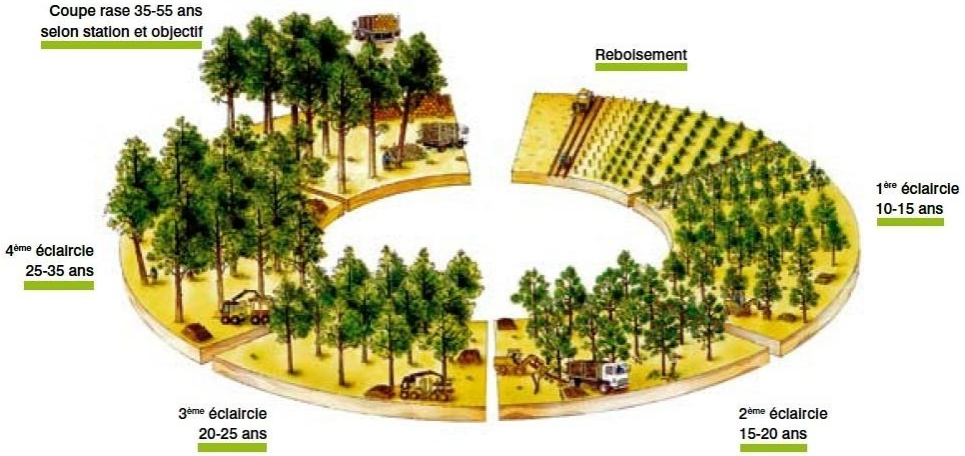


Figure 5 : Schéma de la culture du pin maritime classiquement utilisée dans les Landes (CRPF Aquitaine : Centre Régional de la Propriété Forestière d'Aquitaine).

L'utilisation de variétés améliorées de pins maritimes débute en 1960 à la suite de la mise en place du programme d'amélioration génétique qui avait pour objectif d'augmenter le volume et la rectitude des arbres afin d'accroître la quantité et la qualité de la production de bois du massif (Durel 1992). En comparaison des plants non améliorés, les dernières variétés améliorées commercialisées aurait ainsi gagner 30% en volume (GIS 2002), et la production actuelle de bois dans le massif forestier est estimée actuellement à environ 12 m^3 par hectare et par an (Loustau *et al.* 1999). Aujourd'hui, les conséquences à long terme de l'intensification de la sylviculture dans le massif sont inquiétantes, notamment vis-à-vis des maladies du pin maritime. En effet, les insectes herbivores, tel que les scolytes (*Ips sexdentatus*) et la chenille processionnaire (*Thaumetopoea pityocampa*) par exemple, ou des espèces de champignons pathogènes du pin maritime, telle que l'Armillaire ou le fomès, sont à l'origine d'importantes mortalités pour lesquelles l'homogénéisation de l'espèce plantée, l'homogénéisation des âges des arbres et l'homogénéisation génétique semblent être des facteurs favorables à leur multiplication (Jactel *et al.* 1994 ; Samalens & Rossi 2010). Le cas de l'Armillaire est particulièrement intéressant car supposé autochtone et ayant pu fortement bénéficier de ces changements du paysage comme en témoigne l'augmentation du nombre de signalement depuis trente ans (Aumonier 2007).

IV. L'Armillaire obscure : *Armillaria ostoyae*

1. Description et répartition géographique

Armillaria ostoyae (Romagnesi) Herink, est un Basidiomycète de l'ordre des Agaricales appartenant au genre *Armillaria* qui comprend une quarantaine d'espèces à l'échelle de la planète, dont sept sont actuellement décrites en Europe (Figure 6). Ce champignon fait partie des agents de pourridiés (Encadré 1) et présente un cycle biologique qui associe une phase parasitaire, mais aussi, une phase saprophyte (Figure 8). *A. ostoyae* est probablement l'une des espèces du genre *Armillaria* présentant la plus importante gamme de stratégies biologiques, car décrite comme saprophyte, parasite secondaire et même parasite primaire selon l'hôte et l'environnement sylvicole où elle est présente (Guillaumin & Legrand 2005 ; Marxmüller & Guillaumin 2005).

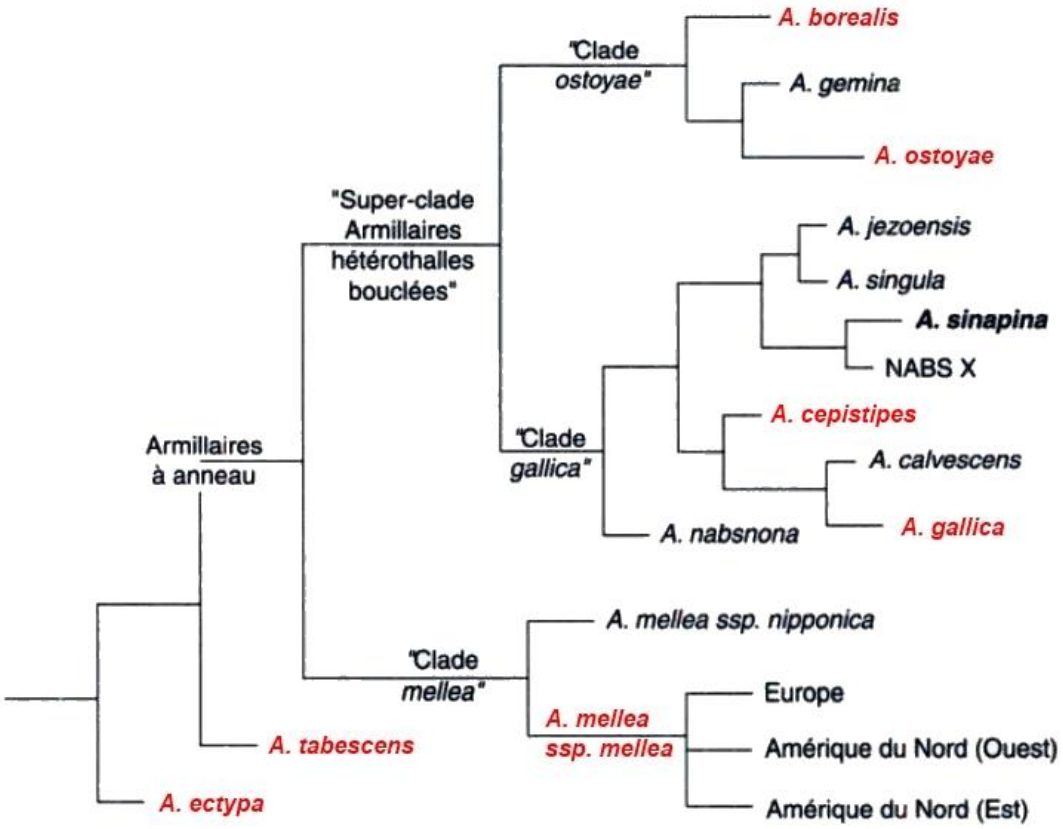


Figure 6 : Phylogénie des espèces d'Armillaire de l'hémisphère Nord (Guillaumin & Bérubé 2005)

Encadré 1

- Agents de pourridiés : Champignons saprophytes facultatifs, responsables de maladies racinaires des végétaux ligneux. L'ensemble des végétaux ligneux est concerné, allant des arbres forestiers (feuillus et conifères), des arbres fruitiers, des arbustes d'ornement, de la vigne et des cultures ligneuses (café et théier par exemple). Les grosses ou moyennes racines sont majoritairement concernées par les infections des agents de pourridiés. Bien que les Ascomycètes constituent une part non négligeable des agents de pourridiés (*Rosellinia necatrix* par exemple), ce sont toutefois les Basidiomycètes qui sont les plus nombreux. Ils sont le plus souvent représentés par les Agaricales et les Polyporales, dont le genre *Armillaria* et le genre *Heterobasidion* sont respectivement les plus étudiés.

A. ostoyae est à l'origine d'importantes mortalités chez les conifères de l'hémisphère nord, notamment en Amérique du Nord, en Europe et même en Asie (Figure 7). En Amérique du Nord, l'aire de répartition d'*A. ostoyae* s'étend de l'extrême-ouest du Canada (Colombie-Britannique ; Morrison *et al.* 1985), jusqu'à l'extrême-est du pays (Ile de Terre-Neuve ; Bérubé 2000), en passant par les provinces des prairies : Alberta, Saskatchewan et Manitoba (Mallett 1992). On retrouve également l'agent pathogène aux Etats Unis d'Amérique, et plus particulièrement dans les états du nord (par exemple le Minnesota (Rizzo *et al.* 1995) et le Wisconsin (Banik *et al.* 1995)) et de l'ouest (Oregon et Washington par exemple (Thies 2001). Les forêts de conifères du Nouveau Mexique (Marsden *et al.* 1993) et du nord du Mexique (Shaw 1989) constituent la limite sud de l'aire de répartition d'*A. ostoyae* en Amérique du Nord. En Europe, *A. ostoyae* est présente dans les forêts de nombreux pays (Guillaumin *et al.* 1993 ; Figure 7B). En Asie (Figure 7C), *A. ostoyae* a été signalée en Chine (Mohammed *et al.* 1994), en Corée (Sung *et al.* 1994) et également au Japon (Ota *et al.* 1998).

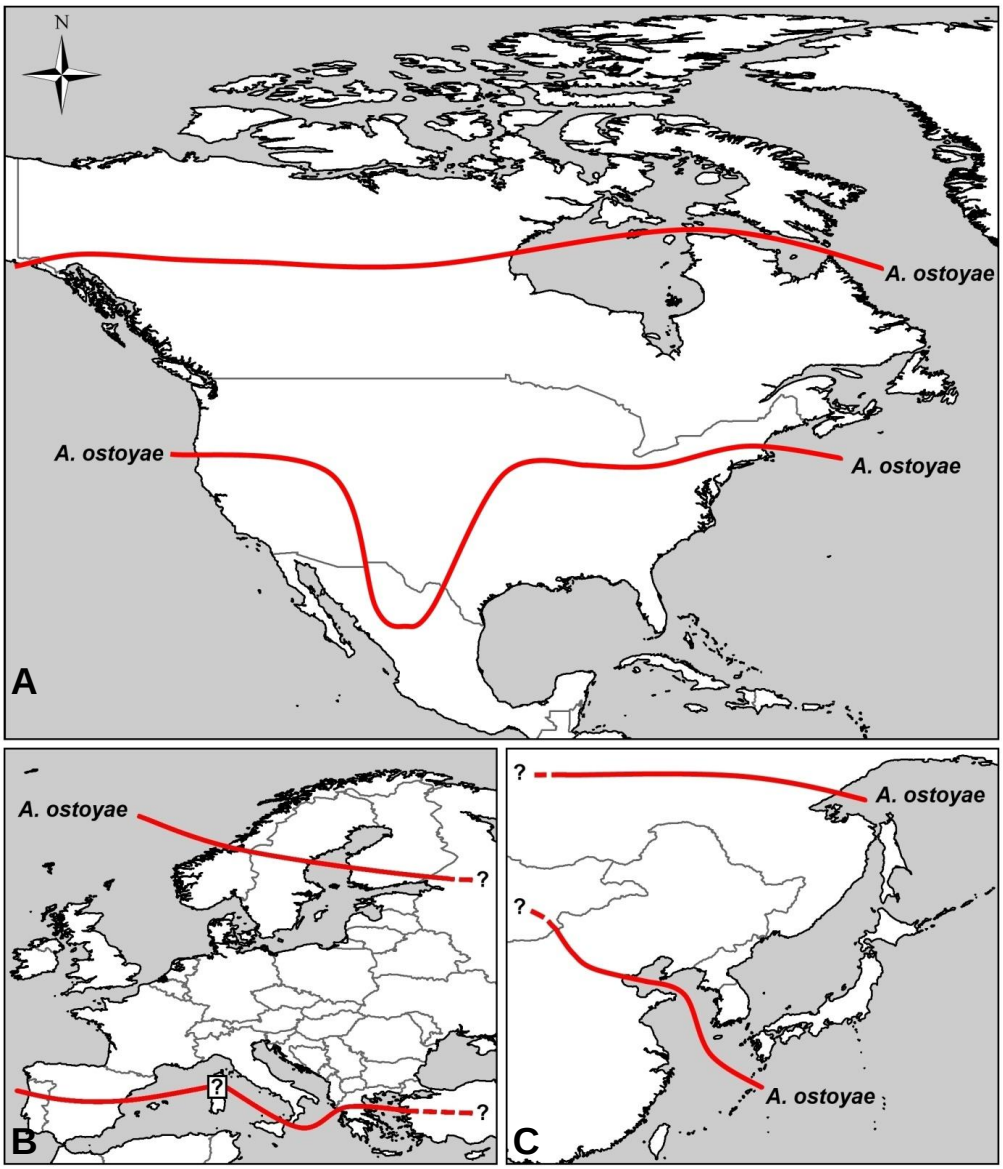


Figure 7 : Cartes des limites nord et sud des aires de répartition d'*A.* ostoyae en Amérique du Nord (**A**), en Europe (**B** ; d'après Marxmüller & Guillaumin 2005) et en Asie (**C**).

2. Cycle biologique

a. Phase parasite

A. ostoyae peut infecter un arbre hôte par les rhizomorphes ou par des contacts racinaires. Les rhizomorphes sont des structures mycéliennes différenciées souterraines (*Rhizomorpha subterranea*). Ces structures, qui sont constituées d'un cordon cylindrique sombre dont le diamètre varie entre 1 et 5 mm, peuvent s'accoler aux racines lignifiées puis, par l'action d'enzymes de dégradation et de forces mécaniques, traverser l'écorce des racines lignifiées et atteindre le cambium (Figure 8 & Figure 9A ; Hartig 1874 ; Zeller 1926 ; Day 1927 ; Rykowski 1975).

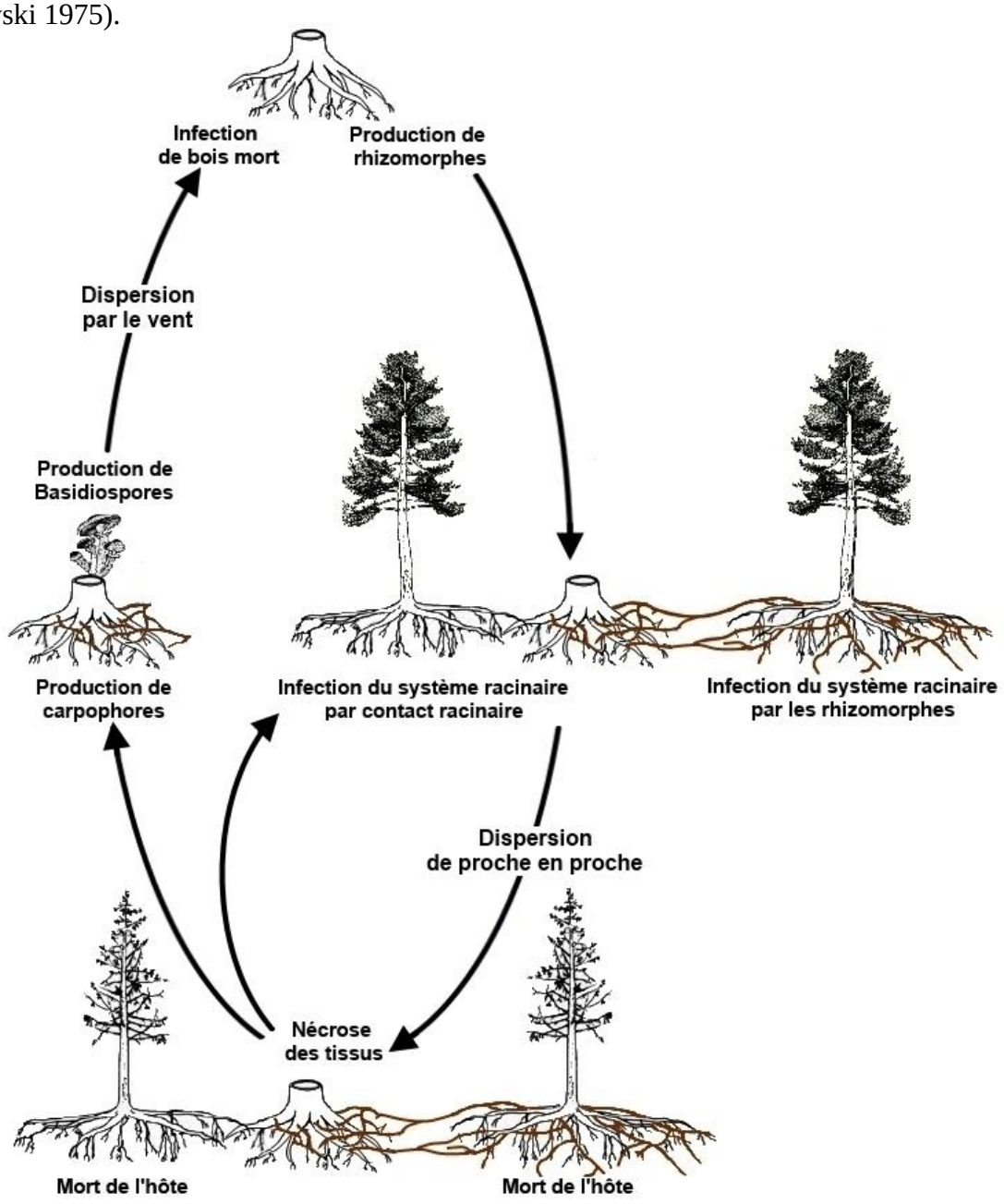


Figure 8 : Représentation schématique du cycle biologique d'*A. ostoyae* (modifié à partir des dessins de V. Fataar, WSL).

L'infection par *A. ostoyae* peut également s'effectuer par simple contact racinaire entre les arbres colonisés par le pathogène et les arbres sains du voisinage (Figure 8 ; Shaw 1980; Lung-Escarmant *et al.* 2003). Dans ce cas, les hyphes indifférenciés traversent les parois des cellules ligneuses et progressent prioritairement dans le cambium. Ce tissu présente de faibles résistances mécaniques, notamment en raison de sa faible composition en lignine, et qui constitue donc une réserve nutritive aisément accessible (Figure 10A ; Allsopp & Misra 1940 ; Guillaumin & Legrand 2005). Suite à l'infection, le mycélium se forme dans la zone cambiale et s'agrège alors en palmettes épaisses (Figure 9B) qui progressent jusqu'à la pointe des racines, mais aussi jusqu'au collet à partir duquel le champignon pourra alors infecter l'ensemble du système racinaire et ceinturer le collet, conduisant progressivement ainsi à la mort de l'arbre.

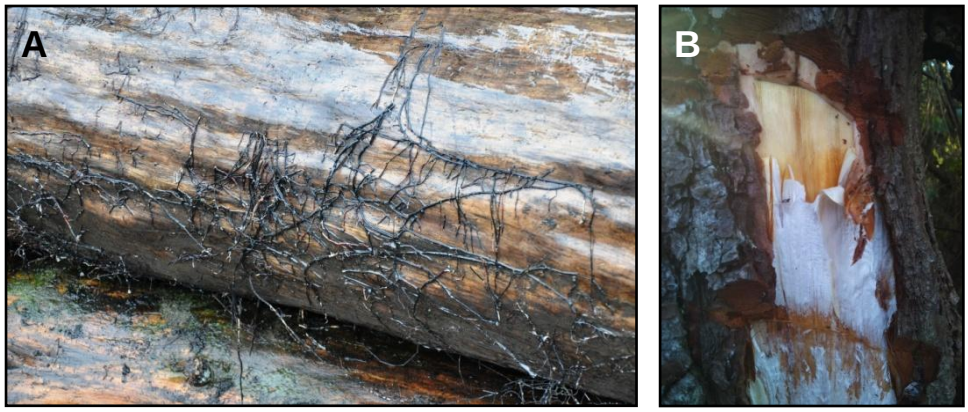


Figure 9 : **A** : Photographie de rhizomorphes d'*A. ostoyae* sur pin maritime (T. de Courcelles) ; **B** : Photographie d'une palmette sous-corticale d'*A. ostoyae* au collet d'un pin maritime.

Cependant, l'envahissement des racines par les palmettes sous-corticales du pathogène peut être stoppé par des mécanismes de défense de l'hôte. En effet, l'hôte, en réponse à l'infection, produit généralement au site d'infection une assise génératrice subérophellodermique formant une barrière imperméable de cellules subérifiées permettant un maintien temporaire de l'infection (Cleary *et al.* 2012). De plus, une production importante de résine par l'hôte au niveau du système racinaire (Robinson 1997), ainsi que la formation de manchon de résine recouvrant les points d'infections (Rykowski 1975 ; Figure 10B), peuvent contenir physiquement ces infections en empêchant la progression du pathogène jusqu'au collet, fatale pour l'arbre. Alors que Rishbeth (1972) a montré l'implication de la térébenthine (composé volatile de la résine) dans l'inhibition de la croissance en culture d'*A. mellea* (*sensus stricto*), il a été suggéré que la résine soit surtout impliquée dans le blocage physique des trachéides (Prior 1975 ; Redfern 1978). L'hôte peut également former des cals en réponse à l'infection ainsi que des racines adventives qui se substitueront aux racines infectées

(Robinson & Morrison 2001 ; Figure 10C). La mise en place de ces barrières histologiques (Figure 10C & D), permet aux arbres de contenir, voire d'éliminer, l'agent pathogène au point d'infection initial dans des "lésions latentes" (Thomas 1934 ; Desray *et al.* 1998 ; Guillaumin & Legrand 2005). Les arbres infectés ne présentent alors aucun signe de dépérissement (Delatour & Guillaumin 1995 ; Banik *et al.* 1995). Dans le cas particulier de l'épicéa commun, ces lésions racinaires latentes peuvent cependant être à l'origine de pourritures de cœur (Nierhaus-Wunderwald *et al.* 2012). En effet, malgré le confinement d'*A. ostoyae* dans quelques racines de son hôte, le champignon peut se propager dans le cœur du tronc et progresser jusqu'à une cinquantaine de centimètres environ au-dessus du sol. Dans ces conditions, l'arbre dont le cambium n'est pas touché, peut alors survivre plusieurs années sans présenter le moindre symptôme de la maladie. En revanche, la pourriture de cœur le fragilise devenant par conséquent, beaucoup plus sensible au vent, notamment lors des tempêtes.

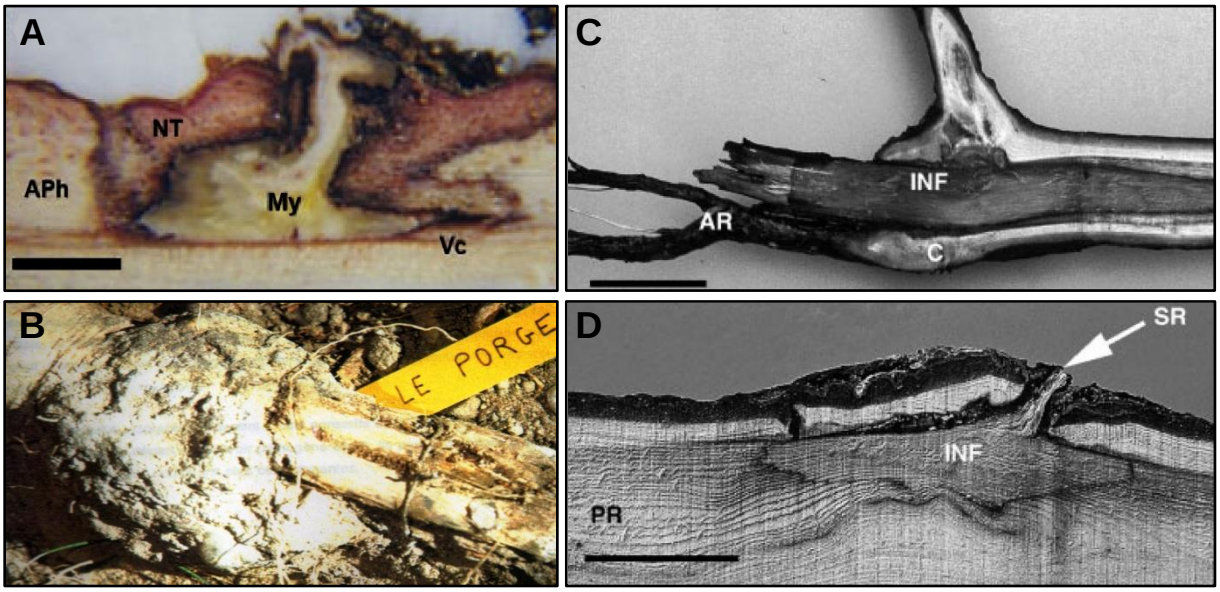


Figure 10 : **A** : Photographie montrant l'invasion par le mycélium d'*A. ostoyae* (My), de l'écorce et du cambium (Vc) d'une racine de douglas (NT : tissus nécrosés, APh : phloème adjacent, barre = 10 mm ; Cleary *et al.* 2012) ; **B** : Photographie d'un important manchon de résine en amont d'une infection par *A. ostoyae* (Robene-Soustrade 1993) ; **C** : Photographie de la formation d'un cal (C) et d'une racine adventive (AR) en réponse à une infection racinaire confinée d'*A. ostoyae* (INF) chez un mélèze (*Larix occidentalis* ; barre = 2 cm ; Robinson & Morrison 2001) ; **D** : Photographie de l'infection par *A. ostoyae* (INF) d'une petite racine secondaire (SR) confinée à la jonction avec la racine primaire d'un douglas (barre = 2 cm ; Robinson & Morrison 2001).

Toutefois, en cas de rupture de l'équilibre établi entre le parasite et l'hôte à la suite d'un stress biotique ou abiotique, l'hôte ne peut plus maintenir les mécanismes de défenses décrits précédemment et, par conséquent, le pathogène poursuit son infection et envahit rapidement le système racinaire jusqu'au collet (Guillaumin & Legrand 2005).

L'influence de l'âge de l'hôte vis-à-vis de la sensibilité de l'hôte à l'Armillaire est incontestable (Robinson & Morrison 2001 ; Lung-Escarmant & Guyon 2004). Les arbres les plus résistants sont ceux d'âge intermédiaire (10 à 20 ans), pour lesquels les mécanismes de défense mis en place semblent mieux contenir la maladie. En effet, contrairement aux hôtes plus âgés, les jeunes plants ne disposent que d'une fine écorce interne (le liber), qui ne leur permet donc pas de mettre en place un nombre suffisant de barrières histologiques pour contenir l'agent pathogène. Ce dernier se retrouve alors très rapidement en contact avec le cambium de l'hôte (Robinson & Morrison 2001). Le faible éclairement dont bénéficient les arbres dominés (Davidson & Rishbeth 1988 ; Wargo & Harrington 1991), et les températures extrêmes ou, tout simplement, non optimales pour la croissance racinaire de l'hôte (Bliss 1946 ; Wargo & Harrington 1991), font partie des autres principaux facteurs de prédisposition à *A. ostoyae*. L'influence de la pollution est plus contestée, mais des fumigations au dioxyde de souffre (SO_2) intensifierait l'infection et les mortalités associées à *A. ostoyae* (Horak & Tesche 1993). L'effet du stress hydrique sur la prédisposition à *A. ostoyae* a également souvent été mentionné à la suite d'événement de sécheresse (Wargo & Harrington 1991). Des essais d'infection en conditions contrôlées ont aussi mis en évidence une mortalité significativement plus forte sur des semis de sapin de Vancouver (*Abies Grandis*) de trois ans soumis à un stress hydrique (Parks *et al.* 1994), mais il me semble que cela ne soit pas toujours le cas (Wahlström 1992 ; Lung-Escarmant *et al.* 2003a). De la même manière qu'un manque d'humidité, un excès peut également contribuer à prédisposer l'hôte, par l'anoxie du système racinaire de l'hôte (Wargo & Harrington 1991). Contrairement au fomès par exemple (Hunt & Krueger 1962 ; Wallis & Morrison 1975), les blessures racinaires ou du collet ne constituent pas de nouvelles portes d'entrée pour l'Armillaire, et ne jouent donc pas de rôle direct sur la prédisposition des hôtes à l'Armillaire. Cependant, elles peuvent faciliter indirectement l'infection par l'agent pathogène, notamment en stressant l'arbre qui doit alors cicatriser ces blessures ou substituer ses racines trop endommagées (Popoola & Fox 1996). Celles-ci peuvent également faciliter la pénétration par les rhizomorphes, qui semble facilitée dans les zones de faiblesse des racines (Desray *et al.* 1998). Enfin, lors de la coupe de l'arbre (Hood & Sandberg 1993 ; Cruickshank *et al.* 1997), les défenses de l'hôte dans les racines s'effondrent et l'Armillaire présent peut alors rapidement coloniser la souche. Cette présence donne un avantage certain à l'Armillaire en comparaison des autres saprophytes du sol ou des

autres champignons colonisateurs du bois disséminés par les spores (Delatour & Guillaumin 1995 ; Legrand *et al.* 1996).

b. Phase saprophytique

Lors de la phase saprophytique, *A. ostoyae* colonise les souches ou les fragments de bois morts présents dans le sol, ce qui lui permet probablement de persister plusieurs dizaines d'années. Cela a été démontré par exemple pour *A. mellea*, dont la durée de vie dans une souche pouvait dépasser les 40 ans (Rishbeth 1972). Cette particularité est d'ailleurs à l'origine de la difficulté pour les sylviculteurs de lutter contre cet agent pathogène dont l'inoculum peut persister dans le sol même en l'absence d'hôte, comme c'est le cas par exemple, lors de la transition entre deux plantations successives (Redfern & Filip 1991). En revanche, les taux de réussite des isolements effectués par Tsoumou-Gavouka (1982) à partir de racines de chêne inoculées par *A. mellea* et enfouies pendant une année dans le sol, témoignent d'un moins bon maintien de l'inoculum dans les racines de faible diamètre, où il semble finir par s'épuiser. Par ailleurs, pour un hôte donné, il n'existe pas de différence entre le bois issu du système racinaire et celui issu de la partie aérienne (Guillaumin & Legrand 2005). Les Armillaires colonisent essentiellement les cellules aux parois peu lignifiées que l'on retrouve dans les rayons médullaires et les canaux sécréteurs de résines (Encadré 2 & Figure 11A ; Robene-Soustrade 1993). La lumière des trachéides est par la suite envahie par les hyphes (Figure 11B ; Robene-Soustrade 1993), et le transfert d'une trachéide à l'autre est alors assuré par des microhyphes qui perforent les parois lignifiées des cellules. Enfin, la lamelle moyenne des trachéides est décollée ce qui provoque la séparation des trachéides (Figure 11C ; Guillaumin & Legrand 2005).

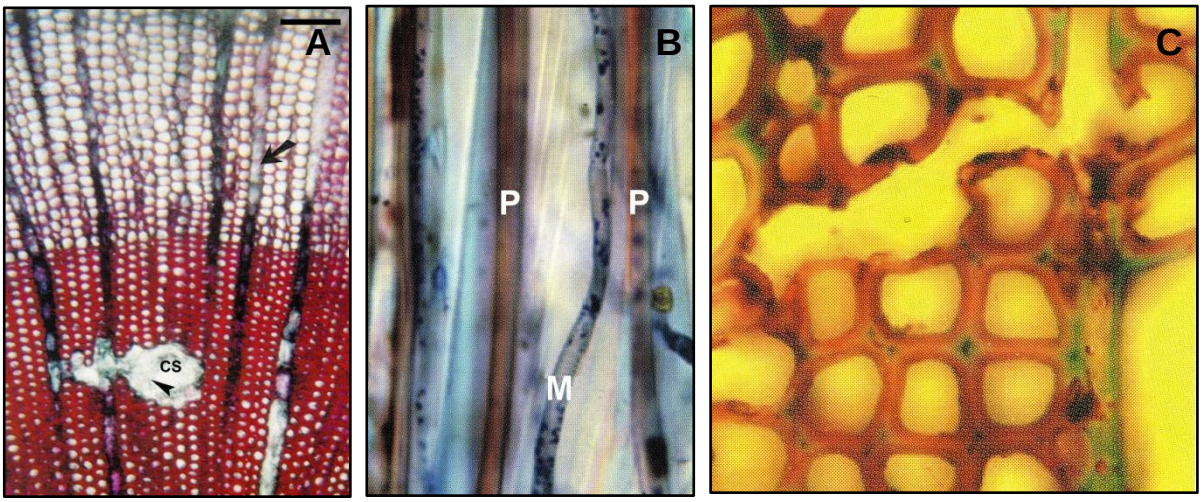


Figure 11 : **A** : Photographie en microscopie photonique, d'un pivot de pin maritime coloré à la phloroglucine (coloration en rouge de la lignine) et au bleu coton, montrant la présence du

mycélium d'*A. mellea* (flèches) dans un canal sécréteur (CS) en coupe transversale et dans un rayon médullaire (Barre = 100 µm ; Robene-Soustrade 1993). **B** : Photographie en microscopie optique, montrant la présence du mycélium (m) d'*A. mellea* dans les trachéides en coupe longitudinale d'un noisetier (K. Rykowski ; Guillaumin *et al.* 2005). **C** : Photographie en microscopie optique, montrant la destruction des lamelles moyennes responsables du décollement des trachéides d'un noisetier en coupe transversale (K. Rykowski ; Guillaumin *et al.* 2005).

Comme pour d'autres champignons lignivores, tel que *Polyporus squamosus* et *Stereum hirsutum* par exemple (Campbell & Munson 1936 ; Lopez-Real 1975), on observe régulièrement de minces et nettes formations sombres dans le bois colonisé par l'Armillaire (Figure 12A ; Campbell 1934 ; Lopez-Real 1975), que l'on appelle "zone-lines" ("black-lines", ou encore "pseudosclérotes"). Ces formations, le plus souvent de forme isodiamétrique, sont constituées d'articles mycéliens mélanisés qui délimitent un volume de bois contaminé par le mycélium indifférencié du pathogène (Figure 12B). Cette délimitation constitue une barrière qui protège partiellement l'Armillaire de la dessiccation et des autres microorganismes antagonistes, ainsi que d'autres espèces d'Armillaire ou même d'autres génotypes de la même espèce par exemple (Hood & Morrison 1984). Le bois contenu au sein d'une zone-line constitue une réserve nutritive disponible pour le pathogène qui est progressivement dégradé sous l'action de complexes enzymatiques (voir chapitre III).

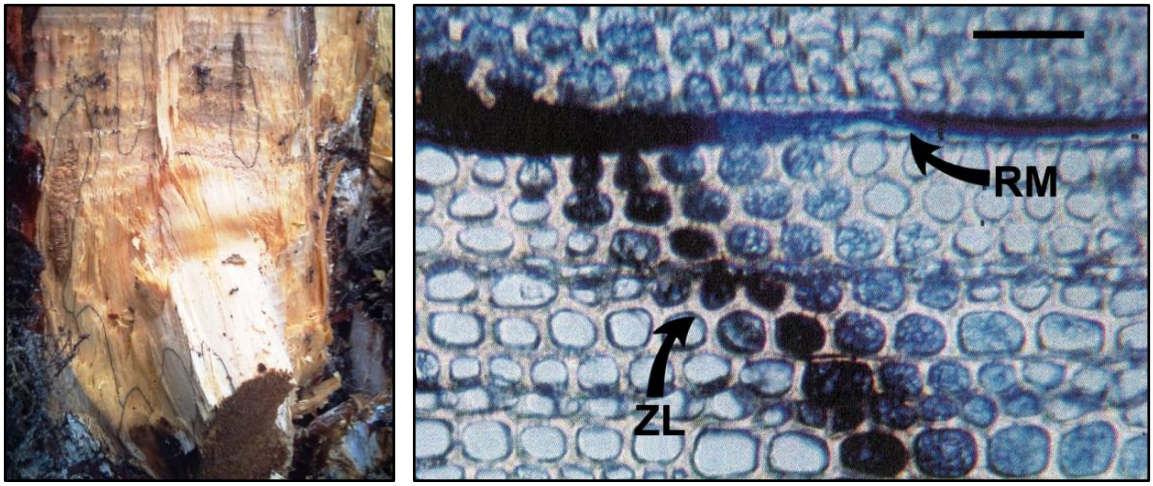


Figure 12 : **A** : Photographie de zone-lines d'*A. ostoyae* au collet d'un pin maritime ; **B** : Photographie en microscopie photonique d'une coupe bûchette de pin maritime, montrant par coloration au bleu coton la présence du mycélium d'*A. mellea* dans les rayons médullaires (RM) et des zone-lines (ZL). (Barre = 100 µm ; Robene-Soustrade 1993).

Cependant, en comparaison d'une grande partie des agents de pourriture blanche, *A. ostoyae* possède des capacités de dégradation du bois relativement faible, suggérant que cette phase n'est qu'une étape de transition vers sa phase parasitaire (Guillaumin & Lung 1985 ; Robene-Soustrade 1993). En effet, la formation d'une réserve d'inoculum importante, par la colonisation des souches et autres fragments ligneux du sol, constitue alors une menace directe pour les hôtes avoisinants.

Encadré 2

- Trachéides : Cellules du xylème ayant un rôle de soutien global des plantes vasculaires. Elles jouent également le rôle de capillaire pour le transport de la sève brute. Elles sont disposées longitudinalement par rapport au sens du tronc et constituées de ponctuations aréolées permettant le transfert de la sève d'une trachéide à l'autre. L'épaisseur des parois des trachéides permet de distinguer le bois de printemps (parois fines permettant un passage important de la sève), du bois d'été (parois épaisses).

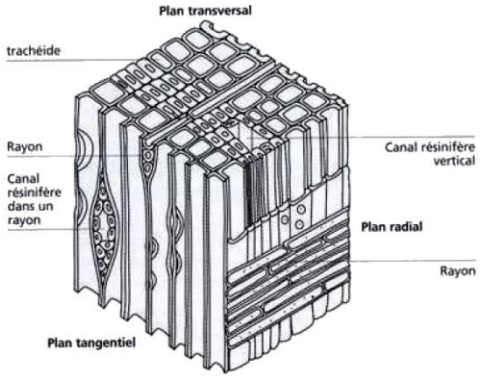
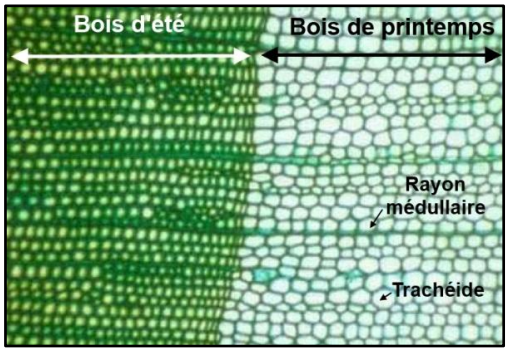


Schéma de la structure des résineux
(Cerig/Pagora, 1996-2008)



Coupe transversale de bois de pin
(Université Pierre et Marie Curie)

- Rayon médullaire : Constitué de cellules de parenchyme toujours orientées dans le sens radial, qui permettent donc la circulation de la sève dans ce sens. Ils sont parfois accompagnés de trachéides horizontales et peuvent également servir de lieu de stockage des nutriments.
- Canal sécréteur (canal résinifère) : Cavité tubulaire disposée dans le sens longitudinal et radial. Ces canaux, qui sont nombreux et régulièrement répartis dans le bois, communiquent entre eux et forment donc un réseau continu.

c. Modes de dispersion

La croissance végétative des rhizomorphes dans le sol et des palmettes dans les racines joue un rôle majeur dans la dissémination du champignon entre les arbres d'une même parcelle. Dans certains cas, un même génotype peut couvrir plusieurs dizaines d'hectares (Zeller 1926 ; Childs & Zeller 1929 ; Smith *et al.* 1992) et même jusqu'à près de 1000 ha (Ferguson *et al.* 2003). Ce mode de dissémination de proche en proche est à l'origine de l'aspect en foyer circulaire propre aux mortalités associées à l'Armillaire et couramment décrites comme "maladies du rond".

A l'échelle d'un massif forestier, c'est la dissémination par le vent des basidiospores haploïdes, issues de la méiose et produites par les carpophores (ou sporophores) qui permet la dispersion de nouveaux génotypes et l'émergence d'un nouveau foyer de mortalité. Les carpophores peuvent émerger en touffes, sur du bois mort colonisé, à l'automne, lorsque les températures sont suffisamment froides et que l'humidité est élevée (Marxmüller & Guillaumin 2005). Les carpophores d'*A. ostoyae* sont de taille variable (8 à 15 cm de diamètre en moyenne) et généralement de couleur brunâtre (Figure 13). Leur chapeau est revêtu d'ornements foncés (squames) et dispose également d'une tache centrale plus sombre. Le stipe cylindrique est également souvent recouvert de ces ornements foncés ainsi que d'un anneau cotonneux blanchâtre à l'épaisseur variable (Marxmüller & Guillaumin 2005).

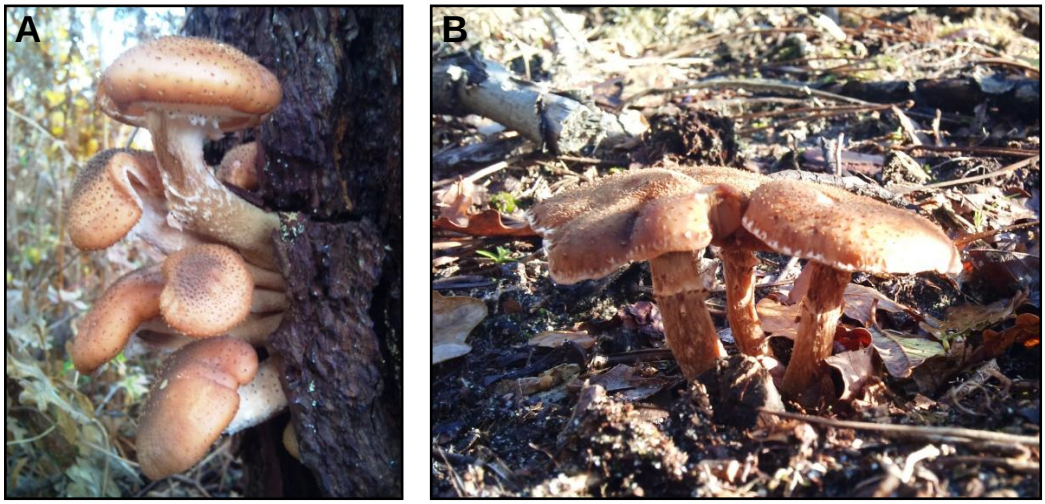


Figure 13 : Photographies de carpophores d'*A. ostoyae* au collet de pin maritime (**A)** et sur racine traçante de pin maritime (**B**).

Cependant, bien que suggéré à plusieurs reprises dans l'installation de nouveaux foyers de la maladie (Rishbeth 1988 ; Legrand *et al.* 1996 ; Prospero *et al.* 2008 ; Dutech *et al.* 2011), l'installation d'un nouveau génotype d'*A. ostoyae* par germination de deux basidiospores

sexuellement compatibles permet l'installation sur un nouveau substrat ligneux favorable ; un mycélium haploïde ne pouvant pas coloniser en général ces substrats (Guillaumin & Legrand 2005). Le type substrat ligneux favorable n'est pas clairement déterminé mais sa présence dans le milieu naturel semble rare (Legrand *et al.* 1996 ; Dettman & van der Kamp 2001). Il pourrait s'agir en priorité de bois mort récent, telles que des souches fraîchement coupées dans un état d'hydratation marqué (Hood *et al.* 2008). De plus, ces basidiospores semblent avoir un pouvoir de germination assez limité comme le montre des expériences en laboratoire où ce taux de germination a été estimé dans ces conditions à 0,001 à 0,0001 % (Rishbeth 1970 ; Swift 1972).

3. Parasite secondaire et primaire des forêts de conifères

a. Parasite secondaire

Dans la plupart des forêts naturelles de résineux, *A. ostoyae* se comporte essentiellement comme un parasite secondaire infectant à la fois les arbres âgés, dominés ou même préalablement affaiblis par d'autres facteurs biotiques ou abiotiques (Davidson & Rishbeth 1988 ; Wargo & Harrington 1991 ; Guillaumin & Legrand 2005). En Cerdagne dans les Pyrénées françaises, de nombreuses mortalités associées à *A. ostoyae* ont été observées dans les peuplements naturels vieillissants de pins à crochets (*Pinus uncinata*) victimes d'importants déficits hydriques (Durrieu *et al.* 1981, 1985). De même, des dégâts associés à *A. ostoyae* sont régulièrement observés dans le Massif central dans les peuplements naturels de pins sylvestres (*Pinus sylvestris*) âgés ou affaiblis par des mauvaises conditions édaphiques et climatiques (Legrand *et al.* 2005). Ce comportement peut également être adopté par *A. ostoyae* en plantations de conifères, notamment lorsque celles-ci sont installées sur des sols peu ou mal adaptés à l'espèce forestière. Cela a été, par exemple, le cas de l'introduction en France du sapin de Vancouver (*Abies grandis*), originaire d'Amérique du nord (Legrand 1998). D'autres situations similaires ont été observées par exemple dans les plantations italiennes de sapin pectiné (*Abies alba* ; Intini 1989), ainsi que dans les plantations françaises de douglas (*Pseudotsuga menziesii* ; Legrand 1997), de pin laricio (*Pinus nigra* spp. *Laricio* ; Villebonne 1999), et même celles de cèdre (*Cedrus altantica*) du sud-ouest (Legrand & Lung Escarmant 2005).

b. Parasite primaire

Dans les plantations monospécifiques de résineux, *A. ostoyae* peut également se comporter comme un parasite primaire capable d'infecter et de tuer son hôte même si ce dernier n'est pas affaibli. Cependant, ce comportement n'est pas toujours clairement déterminé, et dépendrait notamment de l'espèce hôte et du degré d'artificialisation de l'écosystème forestier. De plus, certaines forêts naturelles de l'hémisphère nord comptent parfois plusieurs espèces du genre *Armillaria* ainsi que d'autres champignons de pourriture des racines (*Heterobasidion annosum* par exemple), ce qui complique la distinction et donc l'identification de l'origine des mortalités (Thies 2001 ; Tsykun *et al.* 2011). Enfin, les prédispositions de l'hôte étant souvent mal connues et difficiles à évaluer, surtout dans les forêts naturelles, il est par conséquent complexe de déterminer le rôle exacte joué par *A. ostoyae* pour les mortalités de nombreux écosystèmes forestiers : parasitisme secondaire ou primaire ?

Les mortalités de pin tordu (*Pinus contorta*) pouvant atteindre jusqu'à 20% dans les jeunes régénérations naturelles d'Alberta au Canada, témoignent du parasitisme primaire d'*A.ostoyae* dans les milieux naturels (Blenis *et al.*, 1987 ; Mallett & Volney 1999). Dans les plantations de douglas (*Pseudotsuga menzienii*), de mélèze (*Larix occidentalis*) et de pin tordu de la Colombie-Britannique, *A. ostoyae* adopte également un comportement de parasite primaire en réduisant la croissance de son hôte jusqu'à 50% et en provoquant des mortalités pouvant atteindre 30% des plants dans les forêts à maturités (Bloomberg & Morrison 1989 ; Morrison & Mallett 1996). Dans de telles conditions, son important potentiel d'inoculum peut même localement élargir la gamme d'hôtes du pathogène aux Angiospermes qui ne sont généralement que rarement affectés ou même infectés par *A. ostoyae* en condition naturelle (Guillaumin *et al.* 1993). L'exemple le plus étudié reste toutefois celui du massif forestier des Landes de Gascogne dans le sud-ouest de la France, qui associe à la fois une transformation paysagère importante et une récente intensification des méthodes de sylviculture. Ces facteurs favoriseraient a priori d'une part, l'expansion du champignon pathogène considéré comme autochtone, et d'autre part, des possibilités de sélection des isolats les plus agressifs s'ils sont parallèlement facilement transmis.

4. L'Armillaire dans les Landes

Les premières mortalités associées à *A. ostoyae* sont apparues peu de temps après les plantations massives de pin maritime de la seconde moitié du 19^{e} siècle (Thiveaud 1992 ;

Dupuy 1994 ; Vallauri *et al.* 2012). Parallèlement à l'intensification des méthodes de gestion sylvicoles du pin maritime, le nombre de ces signalements a fortement augmenté au cours des 30 dernières années (Lévy & Lung-Escarmant 1998 ; Aumonier 2007). Du fait que l'essentiel du cycle biologique d'*A. ostoyae* se déroule dans le sol et qu'il soit souvent associé à des scolytes ou d'autre facteurs d'agressions biotiques et abiotiques, les pertes générées par cet agent pathogène sont difficiles à estimer. Ces pertes annuelles ont été grossièrement estimées en 1984 à près d'un demi-million d'euros pour l'ensemble du massif forestier (Lung-Escarmant & Taris 1984). Cependant, l'augmentation des signalements au cours de ces dernières années suggère que les pertes sont actuellement nettement supérieures, qu'elles pourraient également continuer à augmenter dans les prochaines années et affecter plus fortement certaines parties du massif encore peu touchées (Lévy & Lung-Escarmant 1998 ; Aumonier 2007). À la différence d'*H. annosum*, pour lequel il existe un traitement préventif par pulvérisation d'un produit antagoniste (*Phlebiopsis gigantea* ; Korhonen *et al.* 1994), il n'existe pas de méthode préventive efficace contre l'Armillaire. De plus, du fait de la difficulté d'éliminer le champignon d'une parcelle forestière, il est possible que l'intérêt économique des plantations de pins maritimes finisse par diminuer, voir même que le nombre de tiges à l'hectare passe sous le seuil de rentabilité dans les zones les plus touchées comme cela est déjà le cas par exemple dans les plantations de douglas (*Pseudotsuga menziesii*) de Colombie-Britannique (Laflamme & Guillaumin 2005). Comme cela l'a été souligné dans le chapitre II, l'intensification des opérations sylvicoles (élagages, blessures d'engins mécaniques… (Hood *et al.* 2002), contribue à l'accroissement de l'incidence d'*A. ostoyae* en augmentant la fréquence des stress des arbres forestiers. Les incendies, qui détruisent chaque année plusieurs centaines d'hectares de forêts dans le massif forestier landais (environ 1500 ha/an entre 2001 et 2006 ; DFCI Aquitaine 2008), jouent également le rôle de facteurs affaiblissant du pin et sont profitables à l'Armillaire (Hood & Sandberg 1989). D'autres facteurs de risques plus particulièrement associés à *A. ostoyae* et liés aux méthodes de gestion forestière pourraient aussi s'ajouter. En effet, la succession des éclaircies, telles qu'elles sont pratiquées dans le massif forestier, permet la présence quasi constante de souches fraîches et donc des substrats facilement colonisables par l'Armillaire, notamment lorsque des lésions latentes racinaires sont présentes sur les arbres abattus. Les coupes rases, se succédant rapidement, stimulent également le développement de l'inoculum à partir des souches laissées en place et contaminées par des lésions latentes. D'autre part, bien que les multiples opérations sylvicoles réduisent le nombre d'arbres morts sur pied, elles augmentent parallèlement le volume des souches et des branches et donc globalement du bois morts dans le sol des parcelles forestières (Brin *et al.* 2008), favorable au maintien du pathogène à l'état saprophyte.

OBJECTIFS ET METHODOLOGIES

I. Rôle des forêts préexistantes dans l'émergence d'*A. ostoyae*

Les premiers signalements d'*A. ostoyae* sur pin maritime localisés à proximité d'Arcachon (Guyot 1928), la plus forte densité des mentions de la maladie essentiellement retrouvée le long du littoral Atlantique depuis ces 30 dernières années (Lévy & Lung-Escarmant 1998 ; Aumonier 2007), ainsi que la plus forte diversité génétique de l'agent pathogène également mise en évidence à proximité de l'océan (Prospero *et al.* 2008), suggèrent une expansion de la maladie à partir de l'ouest, ce qui pose la question de l'origine de ces premiers foyers. *A. ostoyae* a toujours été considérée comme autochtone dans la région landaise et supposée préexister avant les plantations du 19e siècle (Prospero *et al.* 2008). L'émergence ancienne (environ 7 millions d'année) du clade des Armillaires de l'hémisphère nord (Coetzee *et al.* 2011), ainsi que la répartition paneuropéenne et la capacité d'*A. ostoyae* à parasiter de nombreuses espèces de résineux (pins méditerranéens, ou résineux d'altitude et des forêts boréales), laissent supposer en effet que cette espèce est autochtone dans les forêts européennes. *A. ostoyae* auraient pu se maintenir tout au long du quaternaire sur les boisements résiduels présents dans la région en particulier à l'époque de l'installation humaine dans les fragments forestiers de la côte et le long des cours d'eau (voir chapitre III.2.a). Ces peuplements boisés résiduels auraient alors servi de populations sources pour l'agent pathogène pour coloniser les nouvelles plantations de pin maritime de la seconde moitié du 19e siècle. Cependant, cette hypothèse n'a jamais été testée directement, notamment en raison de la mise en place particulièrement difficile de suivis, dans l'espace et dans le temps, de l'évolution de la maladie. De plus, il est difficile de tirer des conclusions claires sur le rôle joué par ces forêts à partir de ces simples descriptions des signalements de la maladie. En effet, celles-ci ne prennent pas en compte les différences de pressions d'observation du massif forestier, qui pourraient à elles seules expliquer des variations fortes dans la distribution de la maladie. Ces études ne permettent donc pas de représenter exhaustivement la distribution spatiale de la maladie dans les Landes de Gascogne et les conclusions, qui en sont tirées, peuvent alors s'avérer inexactes.

En conséquence, le premier objectif de cette thèse fut de tester l'hypothèse que les forêts préexistantes du sud-ouest de la France, c'est-à-dire les forêts présentent avant les plantations du 19e siècle, seraient à l'origine de l'émergence d'*A. ostoyae* dans le massif forestier des Landes de Gascogne, notamment en jouant le rôle de sources de la maladie. J'ai par conséquent, utilisé des approches épidémiologiques combinées à de récentes numérisations

des cartes anciennes de la couverture des forêts. Les signalements du Département de la Santé des Forêts (DSF) depuis 1989 se focalisant essentiellement sur les gros foyers de la maladie, un inventaire complémentaire de l'Armillaire a, en conséquence, été réalisé sur environ un quart du massif forestier à partir d'inventaires suivant les routes et pistes forestières et considérant toutes les mortalités liées à *A. ostoyae* (grands foyers comme arbres isolés). Ces approches épidémiologiques associées à ces deux inventaires de la maladie, aux méthodes d'échantillonnage et aux échelles spatiales complémentaires, devaient me permettre d'avoir une représentation plus exhaustive de la distribution spatiale de la maladie, d'effectuer une estimation plus fiable des facteurs de risques qui lui sont associés, et de tester l'implication des forêts préexistantes dans l'émergence d'*A. ostoyae* dans le massif forestier des Landes de Gascogne.

II. Variabilité des composantes d'agressivités d'*A. ostoyae* et leurs relations avec les capacités saprophytiques de l'agent pathogène

L'intensification des méthodes de sylviculture au sein de la forêt des Landes de Gascogne, par exemple par l'homogénéisation des hôtes sur près d'un million d'hectare, la forte densité des plants, les nombreuses opérations sylvicoles pouvant être à l'origine d'affaiblissement des arbres, pourraient constituer un environnement particulièrement favorable au développement des isolats d'*A. ostoyae* les plus agressifs. Cependant, aucune étude publiée n'a testé actuellement la gamme d'agressivité des isolats d'Armillaire issus de cette forêt. De plus, de telles modifications du paysage, pourraient également être à l'origine de potentiels compromis dans les différentes stratégies évolutives de l'Armillaire. En effet, lors de la phase parasitaire, le pathogène présent dans le cambium produit en grande quantité au niveau du front de progression de l'infection, des laccases et des polygalacturonases, respectivement impliquées dans la dégradation de la lignine et de la pectine (Robene-Soustrade *et al.* 1998 ; Olson *et al.* 2012). A l'inverse, lors de la phase saprophytique, le champignon libère des Mn-peroxidases, des CM-cellulases et des xylanases qui sont respectivement impliquées dans la dégradation de la lignine, de la cellulose et de l'hémicellulose (Robene-Soustrade *et al.* 1998). On constate alors que les deux phases du cycle biologique d'*A. ostoyae* impliquent à la fois des mécanismes et des complexes enzymatiques différents, suggérant l'existence possible d'un compris évolutif entre ces deux phases importantes du cycle biologique de l'Armillaire. Enfin, sous l'hypothèse que les isolats d'*A. ostoyae* qui présentent une plus forte agressivité auraient donc pu s'installer prioritairement dans les nouvelles forêts, ils seraient à l'origine des nouveaux cas observés dans ces zones. Les forêts préexistantes regrouperaient alors des

isolats au profil d'agressivité très variable et à l'inverse, les forêts plantées après 1857 regrouperaient les isolats les plus agressifs provenant de ces forêts préexistantes.

En conséquence, j'ai dans un premier temps estimé la variabilité du pouvoir pathogène d'*A. ostoyae* vis-à-vis du pin maritime et testé ses relations avec d'autres traits biologiques supposés impliqués dans le parasitisme ou le saprophytisme de l'agent pathogène. Ces traits ont été indépendamment évalués et ont été choisis afin de cibler trois étapes clés du cycle biologique de l'Armillaire : tout d'abord la vitesse de propagation des organes d'infection (rhizomorphes), puis la progression cambiale et enfin la capacité de dégradation du bois mort. La quantification de chacun de ces traits d'histoire de vie permet, en comparant à des expériences d'inoculations contrôlées, d'élucider l'implication de chacune de ces composantes dans la pathogénicité de l'agent pathogène, ainsi que d'éclaircir les relations entre la phase parasitaire et saprophytique du champignon. J'ai, dans un second temps, testé le rôle potentiel de l'agressivité du parasite dans l'émergence de la maladie dans le massif forestier. Pour cela, j'ai déterminé et comparé, par des inoculations artificielles, l'agressivité d'isolats collectés soit au sein de forêts préexistantes, soit au sein des nouvelles plantations de la seconde moitié du 19^{e} siècle.

III. Structure génétique et histoire démographique d'*A. ostoyae*

Bien qu'*A. ostoyae* comporte une phase saprophyte lui permettant de coloniser tout type de bois mort, sa phase parasitaire en revanche concerne essentiellement les conifères, et plus particulièrement, le pin maritime dans le sud-ouest de la France. L'histoire démographique du pathogène serait par conséquent fortement associée à celle du pin des Landes. Une histoire démographique commune (au moins partiellement) entre l'hôte et ses pathogènes associés est fréquemment observée pour les plantes cultivées dont la sélection de génotypes d'intérêts contribue parallèlement à sélectionner les génotypes des agents pathogènes les mieux adaptés à l'hôte (Stukenbrock & McDonald 2008). L'apparition des premiers dégâts d'Armillaire peu de temps après les plantations massives d'un de ses hôtes les plus sensibles lors de la seconde moitié du 19^{e} siècle, ainsi que l'accroissement apparent des dégâts dans le massif forestier depuis ces dernières années (Lévy & Lung-Escarmant 1998 ; Aumonier 2007), laisse supposer un tel processus. Cependant, la forêt de pins des Landes de Gascogne a également subi plusieurs autres périodes de fluctuations démographiques pourraient avoir également un impact sur la démographie du pathogène. En effet, l'histoire démographique de la forêt dans la région, retracée à partir d'études paléopalynologiques, témoigne de variations de la couverture et de la composition des forêts durant le Quaternaire (Frenzel *et al.* 1992 ; Jolivet

et al. 2007), dont la plus importante d'entre elles aurait eu lieu lors du maximum glaciaire (-20000 à -18000 ans) de la dernière glaciation (-110000 à -10000 ans). Ces éventuelles fluctuations démographiques de l'Armillaire auraient donc pu laisser des signatures sur la diversité génétique actuelle du pathogène comme cela l'a, par exemple, été montré sur le mildiou de la vigne (*Plasmopara viticola*) (Fontaine *et al.* 2013). Bien que Prospero *et al.* (2008) ont montré qu'il existait un gradient de diversité génétique d'ouest en est, compatible avec une hypothèse d'expansion des populations d'Armillaire à partir de la côte, affiner ces résultats par un échantillonnage plus dense permet de tester le rôle joué par les forêts préexistantes dans l'émergence de la maladie, notamment si la structure spatiale de l'agent pathogène fait apparaître un gradient depuis une ou plusieurs sources différentes.

Dans un premier temps, j'ai étudié la structure spatiale génétique de l'agent pathogène à partir de l'échantillonnage décrit précédemment (voir objectif 1), et testé si une structuration spatiale pouvait être détectée en lien avec cette expansion à partir de potentielles sources différenciées. Puis, dans un second temps, nous avons testé si l'Armillaire présentait des signatures génétiques associées aux deux événements, à priori les plus majeurs, ayant affecté la démographie de son hôte : c'est-à-dire la réduction des forêts lors du dernier épisode glaciaire, ainsi que la récente expansion suite aux plantations initiées en 1857. Pour cela, nous avons donc testé différents scénarios démographiques de l'évolution de la taille efficace de la population d'*A. ostoyae*, par des approches Bayésiennes approximées (ABC ; Beaumont *et al.* 2002).

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CHAPITRE II

Pre-existing forests as sources of pathogens? The emergence of *Armillaria ostoyae* in a recently planted pine forest

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Fungi are among the principal causal agents of emerging plant diseases, which are a matter of worldwide concern. Changes in land use, such as the expansion of cultivated areas, are implicated in the emergence of fungal diseases, but have been less often reported for native species plantations. In the maritime pine (Pinus pinaster) forest of the Landes de Gascogne (south-western France), pine mortality due to the root rot fungus Armillaria ostoyae (Basidiomycete) has been increasing over the last 30 years. The first cases of this disease occurred in 1920 only few years after a period of rapid major change to the landscape. During the second half of the 19th century the landscape was transformed from marshes to the largest monospecific maritime pine plantation forest in Europe. We carried out two surveys (0.24 and 1 million hectares) of Armillaria root rot disease in the Landes area, to investigate the spatial distribution of pathogen damage and to determine the role of historical factors in the establishment of this pattern. For the two surveys, spatial analyses and generalised linear models revealed a significant effect on the current geographical distribution of A. ostoyae disease of the proportion of pre-existing forest in the vicinity of afforested areas and a significant effect of the proximity of the first forests planted in the coastal dunes. These results suggest that A. ostoyae was commonly distributed in pre-existing forest areas, and that most of these fragments acted as source for the colonisation of newly planted forests. Better predictions on the risk of establishment of new disease foci in this forest area can be achieved from these results.

1. Introduction

Climate change, anthropogenic parasite introductions and changes in land use, particularly those resulting in a loss of biological diversity, have been strongly implicated in the emergence of fungal diseases in recent decades (Anderson et al., 2004). The emergence of fungal pathogens has long been observed in agrosystems, and has been frequently found to be associated with intensification and expansion of crop distribution ranges worldwide (Stukenbrock and McDonald, 2008). The high density and genetic uniformity of cultivated hosts favour the development of a large local inoculum. This, together with the adaptation of pathogens to such homogeneous environments, may account for these emergences (Ennos, 2001; Stukenbrock and McDonald, 2008). Disease emergence associated with changes in forest landscapes has been reported less frequently than disease emergence in crops, possibly because both large forest plantations are more recent and research in agriculture is much more documented than forest research (Ennos, 2001, 2015; Holdenrieder et al., 2004; Pinon and Frey, 1997). Increasing demand for wood is driving an increase in tree plantation worldwide of about 5 million hectares per year (FAO, 2007, 2013). This increase in surface of planted forests will probably lead to larger numbers of disease outbreaks in these ecosystems in the future. Actually, it has been reported in several studies that plantations of native tree species have favoured the spread of endemic pathogens and the constitution of a large local inoculum, by connecting pre-existing small fragmented host populations. For example, this situation is thought to apply to Microcyclus ulei, the endemic agent of South American rubber tree leaf blight, a disease that almost entirely eliminated Brazilian rubber tree (Hevea brasiliensis) plantations at the beginning of the 20th

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century (Lieberei, 2007). Native tree species plantations may have also contributed to the increase in the frequency of fusiform rust (Cronartium quercuum) on pine plantations in the southern USA (Perkins and Matlack, 2002) or Diplodia pinea in Europe (Fabre et al., 2011). The decrease in species diversity in planted forests, favouring contact between host individuals, was also identified as a possible cause of the increase in reported disease incidence, as described for several root rot diseases in North American and North European planted coniferous forests (Gerlach et al., 1997; Korhonen et al., 1998; Morrison et al., 1988; Pautasso et al., 2005). However, in each of these previous studies, the spatial effect of pre-existing forests as a source of inoculum for the closest afforested areas has not been tested.

The pine forest of the Landes de Gascogne, in south-west France, is a large indigenous species plantation of about 1 million hectares. This region underwent major landscape and environmental changes due to the plantation of an intensively managed forest for resin and wood production, which replaced the original moors and marshes. Until the end of the 18th century, the Landes forest consisted mostly of small, fragmented woods (25 ha on average) in the central part of the present-day forest, with a few larger forests stands (280 ha on average) located behind the coastal dunes and in the eastern part of the area (Vallauri et al., 2012). During the second half of the 19th century, attempts were made to develop the local economy, which was previously based on an agro-pastoral system, by draining most of the moors with a wide and deep network of ditches and by planting a forest consisting of a single native species, the maritime pine (Pinus pinaster). In as little as half a century, beginning in the mid-19th century, forest cover increased from 130,000 to 843,000 hectares, including 780,000 ha of mostly continuous maritime pine plantations (Dupuy, 1994; Thiveaud, 1992; Vallauri et al., 2012). This massive afforestation may have provided for native pathogens, such as the root rot fungus Armillaria ostoyae, favorable conditions for outbreaks, firstly by favoring the rise of a large inoculum in a monospecific host population, and secondly by increasing the connectivity between former sparse ancient forest stands.

A. ostoyae (Romagn.) Herink, a basidiomycete from the Agaricaceae family responsible for Armillaria root-rot disease, is thought to be endemic in the Landes de Gascogne. In this area, other Armillaria species are present (A. mellea, A. gallica and A. tabescens), but they rarely infect living maritime pines (Guillaumin et al., 1993). Therefore, Armillaria disease will refer in this study to maritime pine mortality due to A. ostoyae. This disease was first observed on the west coast of France shortly after the major landscape changes described above (Guyot, 1928). A. ostoyae is a pathogen of coniferous species, and it rarely infects other plant species (Guillaumin, 1986; Guillaumin et al., 1993). It spreads principally by an underground dispersal mechanism involving differentiated mycelial structures called subterranean rhizomorphs, which grow from infected pieces of wood towards uninfected roots. The disease can spread over only short distances via this mechanism, but small patches of disease, resulting in the infection of only a few trees, can lead to the disease covering several hectares (Lung-Escarmant and Guyon, 2004; Prospero et al., 2008). This species also produces basidiospores, which are dispersed by the wind after the production of fruiting bodies in the autumn, resulting in the infection of fresh wood a few hundred meters away (Legrand et al., 1996). Genetic studies of A. ostoyae in Landes de Gascogne have suggested that this spore-based dispersal mechanism plays a major role in the establishment of new disease foci (Dutech et al., 2011; Prospero et al., 2008). Although the intensive silviculture performed in the Landes de Gascognes (i.e. in general thinning at 10, 15, 20 and 30 years old and felling at 55 years old (CRPF, 2008)) could favour the colonisation of fresh stumps, its process has not been directly demonstrated in situ for A. ostoyae (Rishbeth, 1988), and its importance in the Landes de Gascogne remains to be quantified. In addition to this parasitic behaviour, A. ostoyae can also behave as a saprophyte, colonising the dead wood in the soil of many hardwood and softwood species (Guillaumin and Legrand, 2005; Legrand and Guillaumin, 1993). This large host range for saprophytism enables the fungus to persist in a forest stand for long periods, even after the death of all hosts in the immediate vicinity (Smith et al., 1992).

Over the last 30 years, the number of reports of the disease in the east of the Landes area has increased and shows a gradient of density increasing from the western coast to the eastern part of the forest (Aumonier, 2007; Lévy and Lung-Escarmant, 1998). In addition, Prospero et al. (2008) showed that genetic diversity of A. ostoyae populations was greater in the west than in the east of the forest massif; a genetic pattern in agreement with a spatial expansion of the population to east. However, these previous inventories may yield an incorrect estimate of the disease distribution. On the one hand, they did not take into account the uneven nature of reports associated with the non-homogeneous distribution of forest areas, the difficulties to access of some parts of the massif (e.g. absence of roads, military areas, etc.), a lower intensity of survey in areas with lower economic values (e.g. north of the massif, border of rivers, natural areas, etc.), and the effect of the host age affecting the ability of A. ostoyae to infect the host (Gibson, 1960; Lung-Escarmant and Guyon, 2004; Redfern, 1978). On the other hand, these inventories were based on reports of high mortality (large disease foci of several hectares) that may bias the estimates of geographical distribution of the disease, if the smallest disease foci (one to few pine trees) were differently distributed. Finally, although the role of pre-existing forests as the source of initial inoculum was assumed to explain this spatial distribution of the disease in the massif (e.g. Prospero et al., 2008), this hypothesis have not been tested yet. The recent digitisation of historical maps of the region before the establishment of the large pine plantations (Vallauri et al., 2012) made it possible to perform this spatial analysis for the first time in this forest area.

Two sources of colonisation were tested in the present study. First, we hypothesized that most of the pre-existing forests were the source of colonisation for the new pine planted areas in the vicinity of these pre-existing forests. This colonisation of new forest areas from a close source should lead to a local aggregation of the disease, still observed today, provided the spread of A. ostoyae is slow in the massif since the first plantations (i.e. the establishment of new disease foci is a rare event, and dispersal of basidiospores is mainly at few hundred meters) (Dutech et al., 2011, unpublished data). Several studies on Armillaria species previously showed that new plantations on pre-existing forests with a previous disease history, showed a greater incidence of the disease in these plantations than those on agricultural land (e.g. Gibson, 1960; Leach, 1937; Swift, 1972). However, at our knowledge, no study has tested the effect of the spatial proximity between pre-existing forests and new planted forests on the distribution of Armillaria disease. Because in the Landes de Gascogne, there is no evidence that A. ostoyae populations were present in most of these pre-existing forests before plantations, this putative origin must be specifically tested. Second, we tested the effect of spatial distances separating these new planted areas from the western coast on the current spatial distribution of A. ostoyae. The pine forests of the coastal dune could have also significantly affected this distribution in the massif because the first large maritime pine plantations, were established in this area, to stabilise the dune and drain water from the marshes (16,000 ha planted in 1840, and 56,000 ha in 1862 over a total area of about 100,000 ha (Goursaud, 1880). Therefore, these first plantations may also have served as sources of inoculum in the first steps of the A. ostoyae range expansion, in addition to the pre-existing forests.

2. Materials and methods

2.1. Datasets analysed

Two complementary datasets were used in this study: a large-scale and a fine-scale dataset.

The large-scale dataset consisted of a set of 547 Armillaria disease observations collected at the scale of the entire Landes de Gascogne forest by the Département de la Santé des Forêts (DSF; Forest Health Department of the French ministry of Agriculture) between 1989 and 2014. The DSF and its network of foresters trained in the identification of forest tree pests have been responsible for the French forest health survey since 1989, and they monitor the changes in and impacts of forest pests. All their observations are collected in a database (http://agriculture.gouv.fr/departement-de-la-sante-des-forets) containing reports of biotic and abiotic damage with an economic impact on the main forest tree species in France. Consequently, DSF agents report only major mortality events and focused more on the forests with high economic values, in the center of the massif. As mentioned in introduction, this uneven reporting effort could bias the study of the distribution of Armillaria disease in the Landes de Gascogne forest. We tried to obtain an exhaustive picture of the distribution of Armillaria disease, by performing systematic reporting in 2012 and 2013, along roads and forest pathways, generating the "fine-scale dataset". The sampled area covered about 240,000 ha and was located to the south of Arcachon (Fig. 1). This area was chosen because it contains both large stands of pines planted in the mid-19th century, and large forests that existed before the period of landscape transformation, at the transition between the coastal dune area and the Landes plateau (Vallauri et al., 2012). By studying this area, we were able to test the hypothesis of a spread of A. ostoyae from the western ancient forest areas to the more recently planted forests in the east. The reporting method consisted of spotting dead or dying pine trees from the car, then stopping and checking the cause of tree death in each case. These road surveys were conducted from November to December, corresponding to the period of fruiting body formation in A. ostoyae. If fruiting bodies were observed or mycelium was found under the bark of a dead or dying pine, the disease was considered to be present and the GPS position was recorded. We avoided repeated observations of the same mortality focus, which would have biased our density estimates, by ensuring that consecutive observations were separated by at least 100 m. This distance was based on the size of most of the disease patches previously observed in the Landes de Gascogne forest (Prospero et al., 2008). The traces of all the roads surveyed were also recorded by GPS, showing that 95% of Armillaria mortality reports were located within 100 m of the surveyed roads.

2.2. Correction for uneven reporting density

For correct estimation of the density of mortality reports, we had to take into account the uneven spatial intensities of reporting for the two areas analysed. We applied the method classically used to estimate disease report density in medical epidemiology, as adapted for forest epidemiology and described by Fabre et al. (2011). This method is based on comparisons of the crude number of records of the disease of interest (i.e. reports of Armillaria-induced mortality) with the crude number of reference records making no mention of mortality by Armillaria. We required a sufficiently large number of reference records to apply this correction, i.e. approximately two to three times more than the crude number of A. ostoyae mortality records (Fabre et al., 2011). For the large-scale dataset, we used as reference reports 1517 reports of maritime pine damages, other than those caused by A. ostoyae, which were evenly distributed throughout the Landes forest. Indeed, reports associated with biotic or abiotic damages showing a strong spatial aggregation (due for example to Heterobasidion annosum) and/or limited to some yearly outbreaks (for example reports of Thaumetopoea pityocampa) may bias the estimated spatial pattern (Fabre et al., 2011). For the fine-scale dataset the references reports were created in silico, and corresponded to points where A. ostoyae disease was not observed in maritime pine forests along the surveyed roads. Fifteen thousand points were drawn randomly from a uniform distribution over the entire study area. A land-use map of France (Corine Land Cover 2006 see Büttner et al., 2012) and recent aerial and satellite photographs (2011–2013, IGN-French National Institute of Geographic and Forest Information and DigitalGlobe Inc.) were then used. Only those points falling in currently existing pine forest, within 100 m of the roads which were surveyed, and distant to at least 100 m to any other report, were retained. We simulated 584 reference points by this method.

2.3. Spatial pattern analysis

For each dataset, we performed a geostatistical analysis of the distribution of A. ostoyae, based on estimates of the semivariance of the presence and absence of Armillaria disease between pairs of locations (Cressie, 1993). Previous descriptions of the spatial distribution of Armillaria in the Landes de Gascogne suggested that a non-stationary model would be appropriate in this case, as the mean mortality frequency was not constant across the entire area (see for example Aumonier, 2007). We fitted a first-order polynomial to the data, to eliminate regional trends. Under the hypothesis of a slow spread of A. ostoyae from pre-existing sources, we expected a spatial clustering of the disease within and in the close vicinity of these sources, associated with lower semivariance for pairs of points separated by shorter spatial distances. Edge effects were minimised by excluding the largest distance classes containing too few pairs from the semivariograms, as classically done for such analyses. We produced the semivariogram by calculating the mean semivariance for 40 distance classes between pairs of points, separated by 1000 m, with 2654–20,657 pairs per distance class for the large-scale dataset, and 2862–11,736 pairs per distance class for the fine-scale dataset. The degree of spatial clustering of the disease was estimated by determining the distance separating pairs of locations at which the value of the semivariogram did not differ significantly from that expected for a random uniform distribution of the disease. This distance, called the range, was obtained for the two analyses by fitting the best spherical, Gaussian or exponential variogram model to the data, by minimising the least-squares residuals. Semivariogram analysis was performed with the gstat package (Pebesma and Graeler, 2015) available in R 2.15.1 statistical software (R Core Team, 2012).

2.4. Estimation of the distribution of A. ostoyae disease

A nonparametric kernel estimation method was used to estimate the local density of A. ostoyae disease and reference records, for each point of a geographic grid (Wand and Jones, 1995). We used a 310 Â 310 m grid for the large-scale dataset and a 75 Â 75 m grid for the fine-scale dataset. A quartic kernel with a bandwidth of 3.3 km was used to estimate the densities of both A. ostoyae disease and reference records. The most appropriate bandwidth was estimated by least-squares cross-validation (Wand and Jones, 1995). A standardised report rate (SRR) was then calculated as the ratio between the density of A. ostoyae records (Arm_report) and that of reference records (Reference_report), standardised with the ratio of the total number of A. ostoyae records and the total number of reference records

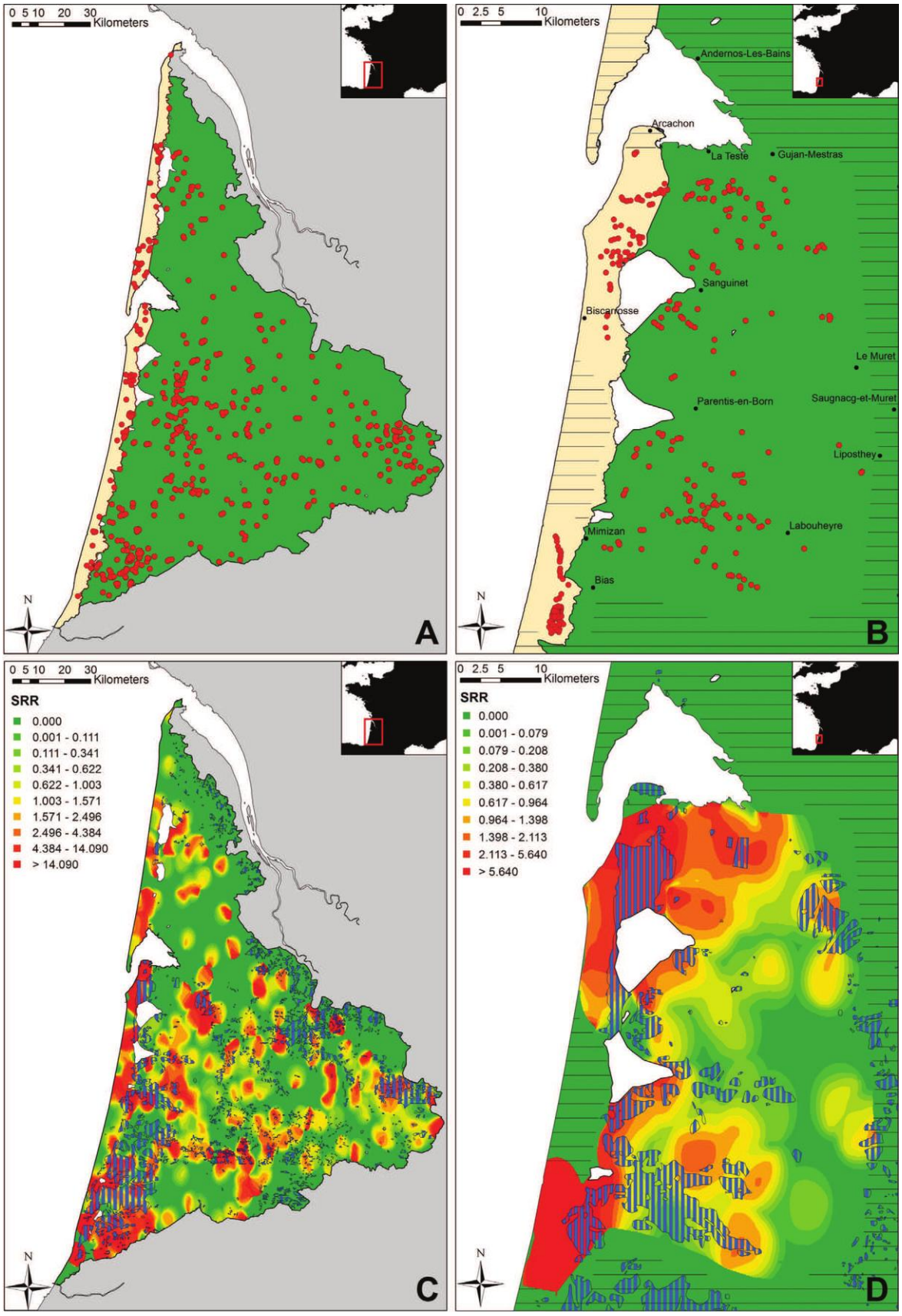

Fig. 1. Reports of A. ostoyae disease on maritime pine in the Landes forest of Gascogne. (A) Large-scale dataset; (B) fine-scale dataset. The Atlantic dune areas are indicated in yellow, and unsurveyed areas are indicated by horizontal hatching. A. ostoyae mortality reports are indicated by red dots. Modelled distribution map of A. ostoyae disease on maritime pine in the Landes forest of Gascogne. (C) Large-scale dataset; (D) fine-scale dataset. High intensities of the disease are indicated in red and low intensities are shown in green. The pre-existing forest areas (Cassini) are indicated by blue hatched areas. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(mean_report_rate), as described by Fabre et al. (2011). SRR was therefore estimated as follows, for each point on the grid:

SRR ¼ ðArm_reportÞ=ðReference_report Â mean_report_rateÞ

We generated a map to identify the areas with the highest potential bias in these estimates (for example due to a low density of points in some parts of the studied area). The potential bias was estimated by bootstrapping and was based on a comparison between the observed SRR map and the mean SRR for 100 simulated datasets. Simulations were performed by assigning a value indicative of the presence (1) or absence (0) of A. ostoyae disease to each point, according to a binomial distribution. The probability value used was that of observing Armillaria disease at a given location and was calculated as the ratio of A. ostoyae report density to reference report density at the location concerned. SRR was determined with the splancs package of R (Bivand, 2015).

2.5. Risk factors associated with Armillaria disease

We investigated the effect of distance to the Atlantic dune, the proportion of pre-existing forest areas (i.e. areas already forested in 1770 on the Cassini map) in the neighbourhood of each disease/reference record, and of host age on the likelihood of a record being an A. ostoyae disease record (1) or a reference record (0).

We estimated the distance to the dune using the limits of the Atlantic dune zone defined by the French National Forest Inventory (Fig. 1). A distance of zero was attributed to records of Armillaria and reference records located within the dune area.

The presence of pre-existing forests in the neighbourhood of observations was assessed with the recently digitised Cassini maps delimiting forest cover in France during the second half of the 18th century (Vallauri et al., 2012). In the studied area, the map was established between 1760 and 1788 (sheets 72, 73, 102 to 107 and 135 to 139), mostly around 1770 at the sampled points. This map, published and digitised at the 1:86,400 scale, is a reduction of the "Guyenne" map, initially surveyed at the 1:43,200 scale. The proportion of pre-existing forests was calculated for a circular buffer zone around each A. ostoyae disease or reference record, for both datasets, with ArcGIS ArcView 10.2 software (ESRI, Redlands, CA, USA). The radius of the buffer, 3 km, was chosen so as to fall into the main dispersal range of various fungi (Malloch and Blackwell, 1992; Stenlid et al., 1994; Power et al., 2008) and in the dispersal range of A. ostoyae inferred from spatial genetic analysis (Dutech et al., 2011, Dutech et al. unpublished data). This choice was also validated by the results of the geostatistical analysis (see below).

A significant proportion of the large-scale dataset (56.8%) contain information about the age of the affected host, making it possible to test the effect of this factor on the probability of detecting the mortality in a subset of this dataset. However, in the fine-scale dataset, tree age was unknown for the disease records and the reference points. Consequently, we estimated tree age with the canopy height model (CHM) developed by IGN. This method uses Pleiades stereo image matching, and calculates the height of the canopy by comparing the digital surface and terrain models (Bélouard et al., 2015). Canopy height was estimated over an area corresponding to 27.6% of the area of the fine-scale study, with Pleiades images acquired in 2012. We then used the estimated height of the canopy and the positive correlation between the height and age of pine plantations (R^2 =0.803, P-value <0.001, Fig. S1) which were recorded for 1801 stands of the large-scale dataset, in order to infer the age of each stand containing a disease or reference point. We compensated for slight inaccuracies in the georeferencing of the CHM used and the GPS positioning of field observations, by attributing to each point (disease or reference record) the maximum canopy height calculated by the model in a circular buffer zone with a radius of 5 m around the report and located within the same stand as the point according to the cadastral map (IGN BD parcellaire 2013).

A first multivariate generalised linear model, with a binomial error distribution, was used to investigate the effects of proximity to pre-existing forest fragments in the vicinity, distance to the dune and the interaction of the two factors, on the Armillaria disease record. The percentage of pre-existing forest fragments and the distance to the dune were not correlated in the large-scale dataset (R^2 =0.001, P-value =0.24), but the correlation coefficient between these two variables was significant for the fine-scale dataset (R^2 =0.081, P-value <0.001). Host age was not incorporated into this first multivariate model because data were available for only 56.8% of the large-scale dataset and could be estimated for only 27.6% of the fine-scale dataset due to the spatial limits of the CHM. However, to test whether host age could affect the results, we used a second multivariate generalised linear model with a binomial error distribution, on a part of the data. All the analyses were performed separately for the large-scale and fine-scale datasets. Odds ratios (OR) were estimated, to assess the risk of damage associated with the explanatory variables. The OR reflects the increase in the risk of a maritime pine being infected with the pathogen for a given change in the independent variable. It was calculated for each variable shown to have a significant effect on disease occurrence. The risk factor was determined with the questionr package of R (Barnier et al., 2015). Finally, in order to visualize the potential effects of the pre-existing forest areas in the vicinity, the distance to the dune and the host age on the disease report frequency, the mortality (1) and reference (0) records were plotted against each of these factors for both datasets, and a loess smooth curve (span =0.6; Cleveland and Devlin, 1988), performed in R, was fitted to the data.

3. Results

3.1. Disease distribution

In the fine-scale dataset, 345 Armillaria mortality foci were observed, whereas 147 disease records were reported in the same area in the large-scale dataset. Greater proportions of Armillaria reports (21% and 27% for the large-scale and the fine-scale datasets, respectively) than reference (11% and 23% for the large-scale and fine-scale datasets, respectively) were also observed in pre-existing forest areas for both datasets.

The estimated semi-variance of the presence/absence of A. ostoyae disease steadily increased with the geographical distance separating the observations for the large-scale and fine-scale datasets (Fig. 2). This semivariogram shape indicated a non uniform distribution of the disease. The spherical models provided the best fit to the variograms for the two spatial scales. The range (i.e. the distance at which the semivariance reached the plateau, and for which the observed value between two points was considered independent of the spatial distance) was 7.5 km for the large-scale dataset and 3.1 km for the fine-scale dataset (Fig. 2). A nugget effect (i.e. the estimate of the minimum semivariance for two observations at the same location) was also observed for both datasets (Fig. 2).

The two maps inferred by kernel estimation indicated a high degree of spatial variability of the SRR (i.e. A. ostoyae disease reporting intensity in the forest; Fig. 1C and D). High SRR values were more frequent on the coast, for both datasets. However, several high-intensity patches were also observed in the central part of the Lande de Gascogne forest. This patchy structure is consistent with the variogram analysis. In addition to this east–west signal, areas of high A. ostoyae disease reporting intensity were also found

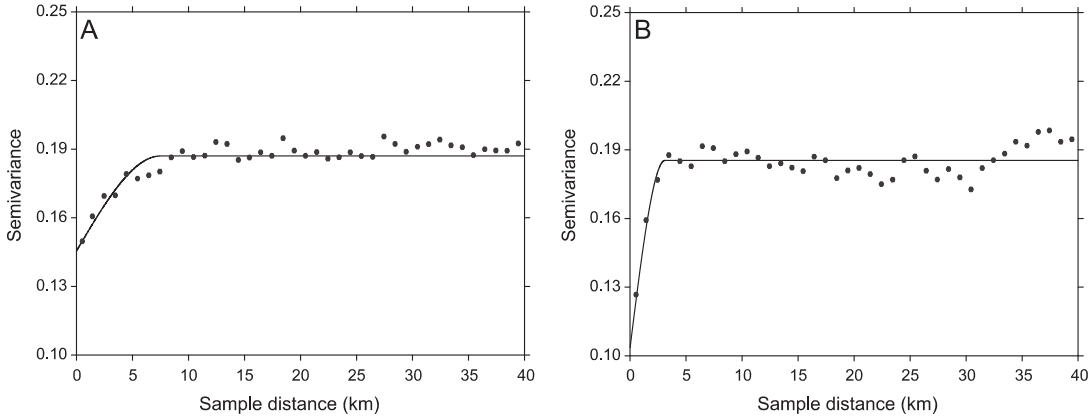

Fig. 2. Semivariogram of A. ostoyae disease (points) and fitted spherical model (lines). (A) large-scale dataset; (B) fine-scale dataset.

Table 1
Odds ratios for the first multivariate model explaining the effects of proportion of pre-existing forest fragments in the vicinity, distance to the dune and factor interaction on A. ostoyae disease occurrence in the Landes de Gascogne (south-west France).

Dataset	Factor	Odds ratio (confidence limits)	P-value
Large-scale	Proportion of pre-existing forest areas in a 3 km buffer area (per 1%)	1.022 (1.015–1.028)	<0.001
	Distance to the Atlantic dune (per 10 km)	0.921 (0.883–0.959)	<0.001
	Proportion of pre-existing forest areas / distance to the Atlantic dune	1.000 (0.999–1.001)	0.82
Fine-scale	Proportion of pre-existing forest areas in a 3 km buffer area (per 1%)	0.986 (0.977–0.995)	0.003
	Distance to the Atlantic dune (per 10 km)	0.184 (0.134–0.249)	<0.001
	Proportion of pre-existing forest areas / distance to the Atlantic dune	1.013 (1.002–1.023)	0.02

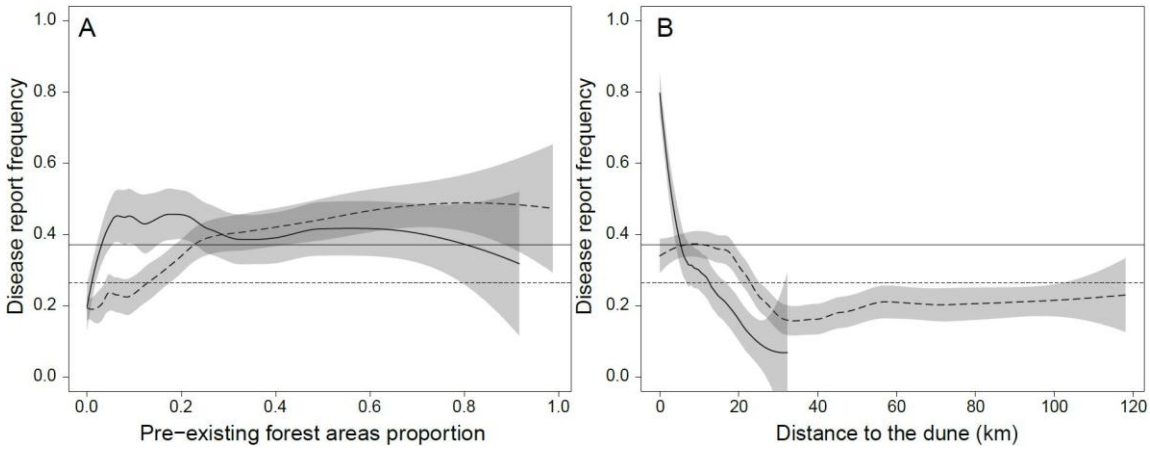

Fig. 3. Frequency of A. ostoyae disease reports (i.e. loess curve (span =0.6) fitted to the Armillaria disease and reference reports), as a function of the proportion of pre-existing forest area in a 3 km buffer area (A), as a function of the distance to the Atlantic dune (B). Horizontal line: mean number of A. ostoyae reports over the surveyed area. Black dashed lines for the large-scale dataset and black solid lines for the fine-scale dataset. The grey areas indicate the confidence intervals (95%).

within or close to pre-existing forest areas (Fig. 1C and D). The relative potential bias in SRR maps (Figs. S2 and S3) revealed a few unreliable areas with a potential bias of the same order of magnitude as the SRR, mostly located in areas with very low SRR values. These areas corresponded mostly to the boundaries of little-sampled areas or areas that were not sampled at all.

3.2. Risk factors associated with the presence of Armillaria disease

For the large-scale database, the first logistic regression analysis showed that both the proportion of pre-existing forest area in the vicinity of an observation and its distance to the coastal dune had a significant effect on the frequency of Armillaria disease reports (Table 1). There was no significant interaction between these two factors (Table 1 and Fig. 4A). The odds ratio associated with the percentage of pre-existing forest was significantly higher than one and indicated that the risk increased by a factor of 1.022 for every 1%increase of surface in this area (Table 1). Consistent with this finding, the frequency of records of Armillaria disease was minimal in the absence of pre-existing forest in the vicinity, with values of 0.19, increasing to a mean of up to 0.48, when the proportion of pre-existing forest exceeded 0.6 (1700 ha) (Fig. 3A). For such high values of the proportion of pre-existing forest in the close vicinity, the observed frequency of Armillaria mortality for all observations was significantly higher than would have been expected for a randomly distributed disease. The risk of disease

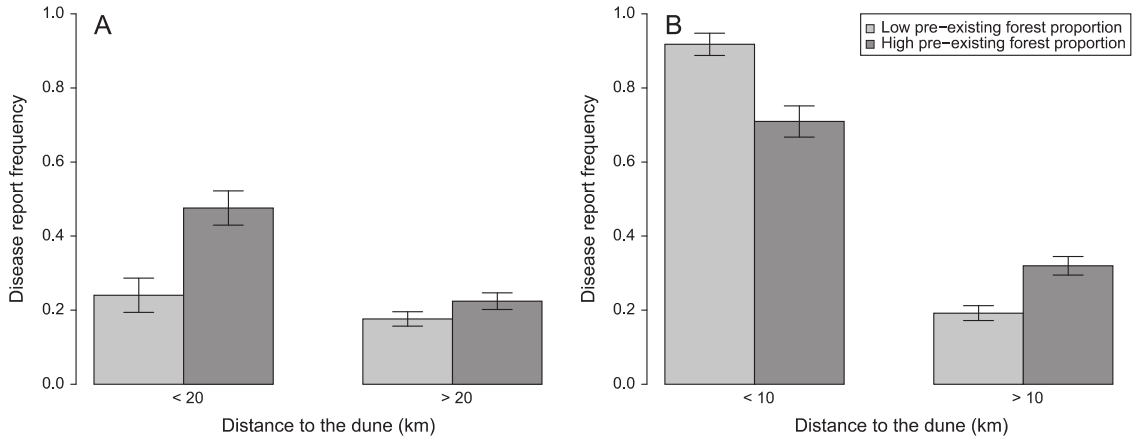

Fig. 4. Frequency of A. ostoyae disease reports according to the factors included in the multivariate model. (A) Large-scale dataset; (B) fine-scale dataset. The two factors were each separated into two classes, with the median value as the cut-off point. Pre-existing forest area proportion lower (light grey, median of 0.01 for the large-scale dataset and 0.13 for the fine-scale dataset) or higher (dark grey) than the median. The vertical bars represent the standard error.

Table 2
Odds ratios for the second multivariate model explaining the effects of proportion of pre-existing forest fragments in the vicinity, distance to the dune, factor interaction and host age on A. ostoyae disease occurrence in the Landes de Gascogne (south-west France).

Dataset	Factor		Odds ratio (confidence limits)	P-value
Large-scale	Proportion of pre-existing forest areas in a 3 km buffer area (per 1%)		1.034 (1.024–1.046)	<0.001
	Distance to the Atlantic dune (per 10 km)		0.970 (0.921–1.020)	0.24
	Proportion of pre-existing forest areas / distance to the Atlantic dune		0.998 (0.996–1.000)	0.03
	Age classes:	0–5 years old/5–10 years old	0.784 (0.528–1.165)	0.23
		0–5 years old/10–22 years old	0.483 (0.307–0.753)	0.001
		0–5 years old/22–60 years old	1.332 (0.930–1.917)	0.12
		5–10 years old/10–22 years old	0.616 (0.400–0.939)	0.03
		5–10 years old/22–60 years old	1.700 (1.221–2.378)	0.002
		10–22 years old/22–60 years old	2.759 (1.881–4.107)	<0.001
Fine-scale	Proportion of pre-existing forest areas in a 3 km buffer area (per 1%)		1.000 (0.989–1.020)	0.61
	Distance to the Atlantic dune (per 10 km)		0.116 (0.047–0.266)	<0.001
	Proportion of pre-existing forest areas / distance to the Atlantic dune		1.028 (1.008–1.048)	0.005
	Age classes:	0–5 years old/5–10 years old	2.741 (1.040–7.436)	0.04
		0–5 years old/10–22 years old	0.417 (0.168–0.994)	0.052
		0–5 years old/22–60 years old	0.426 (0.156–1.100)	0.08
		5–10 years old/10–22 years old	0.152 (0.054–0.399)	<0.001
		5–10 years old/22–60 years old	0.155 (0.051–0.438)	<0.001
		10–22 years old/22–60 years old	1.021 (0.478–2.149)	0.96

decreased significantly with increasing distance to the dune (Fig. 3B), with the lowest risks of disease observed 30 km from the coastal dunes.

For the fine-scale analysis, a similar effect of distance to the dune on the frequency of disease reports was observed with the first multivariate generalised linear model, but no relationship was observed with the proportion of pre-existing forests (Table 1, Fig. 3B and A). The relationship between the frequency of disease reports and areas of pre-existing forest depended on the distance to the dune area with a significant interaction between the effects of the distance to the dune and the proportion of pre-existing forest (Table 1). We investigated this interaction in more detail, by separating our reporting area into two parts: one close to the dune area (<10 km) and another farther away (>10 km). Close to the dunes, the frequency of the disease was minimal when the pre-existing forest area was maximal (Fig. 4B). Conversely, far from the coast, disease frequency was maximal when pre-existing forest area was maximal. However, independently of the proportion of pre-existing forest in the immediate vicinity, the frequency of Armillaria disease was always higher within or close to the dune (Fig. 4B).

The second multivariate generalised linear model also revealed, a significant effect on the frequency of Armillaria disease reports of the proportion of pre-existing forest in the vicinity for the large-scale (odds ratio =1.034, P-value <0.001) and of the distance to the dune area for the fine-scale datasets (odds ratio =0.116, P-value <0.001; Table 2). This second model showed a significant effect of host age on the frequency of A. ostoyae disease reports. The analysis revealed a significantly higher frequency of A. ostoyae disease report for 5- to 10-year-old trees than for 10- to 22-year-old pines (P-value =0.02 and P-value <0.001 for the large-scale and the fine-scale dataset, respectively). The same effect was observed for the youngest pines but only for the fine-scale dataset (<5 years old; P-value =0.04; Fig. 5 and Table 2). For, the large-scale dataset, a significantly higher frequency of A. ostoyae mortality reports was observed for trees of more than 22 years of age than

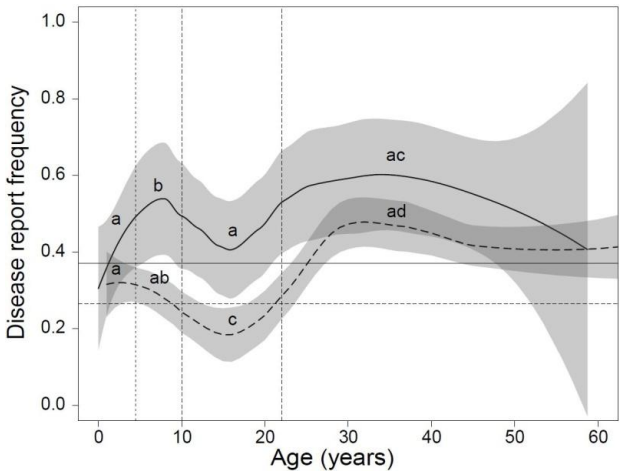

Fig. 5. Frequency of A. ostoyae disease reports (i.e. loess curve (span =0.6) fitted to the Armillaria mortality and reference reports) as a function of host age. Horizontal line: mean number of A. ostoyae reports over the surveyed area (black dashed lines for the large-scale dataset and black solid lines for the fine-scale dataset). Vertical black dashed line: delimitation of age classes. Different letters denote significant differences in disease report frequency between age classes within a dataset.

for 10- to 22-year-old pines, but not for the fine-scale dataset (P-value <0.001 and P-value =0.96, respectively; Fig. 5 and Table 2).

4. Discussion

Consistent with the location of the first historical reports of the disease on the coast (Guyot, 1928), the previous inventories (Aumonier, 2007) and studies of population genetics (Prospero et al., 2008), we found at both the spatial scales investigated, a significant effect of the proximity of the first forests planted on the coast on the current geographical distribution of A. ostoyae disease. The presence of pre-existing forest areas in the vicinity also had a significant effect, but only for the large-scale dataset. For the fine-scale dataset, for which the presence of forest areas in the vicinity and the proximity to the coastal dune interacted, the close proximity of coastal dune could mask the effect of pre-existing forest areas on the occurrence of Armillaria disease. This result suggests that near the coast, the effect of the oldest part of the massif encompassing both all the large pre-existing forests in the west (Fig. 1) and the first forests planted on the dunes was more important than the surface of pre-existing forests in the radius of 3 km of a disease foci. This effect of the surface of pre-existing forests is probably more difficult to detect than the effect of the distance to the coast, possibly due to the random nature of dispersal and the likely absence of the fungus from some of the pre-existing forest fragments. The Cassini maps, which underestimated the forest areas (Vallauri et al., 2012), did not allow to test exhaustively the effect of the proportion of all pre-existing forest areas. In addition, the Cassini maps do not indicate the tree species composition of these forests, but many of them probably consisted of mixtures of pine and hardwood species (e.g. Quercus sp., Betula sp. and Salix sp.) (Dupuy, 1994; Thiveaud, 1992), resulting in heterogeneous host availability for A. ostoyae. In these pre-existing forests, in which pine was less predominant than in modern planted forest stands, A. ostoyae populations probably competed with other saprophytes and was probably less present than today, as observed in some eastern European virgin forests (Tsykun et al., 2011). Moreover, frequent extinction events may have occurred in A. ostoyae populations in the smallest pre-existing forest fragments, limiting their role as sources of inoculum, as suggested by the lower disease occurrence in the smallest fragments of pre-existing forests (i.e. fragments <100 ha, 0.005 ±0.003/100 ha) than in the largest (i.e. fragments >100 ha, 0.024 ±0.008/100 ha). However, despite a weaker effect of the proportion of pre-existing forest in the vicinity than of distance to the coast, we were able to demonstrate a positive effect of the proportion of these pre-existing forest fragments in the vicinity on the mortality caused by Armillaria at the scale of the Landes massif (large-scale dataset), independently of distance to the coast. This finding suggests that the reported large disease foci in the eastern part of this area are more strongly associated with local dispersal from pre-existing forests than rare long-distance dispersal events from the first forests planted on the coast. Furthermore, the analysis at a fine spatial scale shows that this correlation can be detected for locations further from the coastal dunes (i.e. at distances of more than 10 km), at which the effect of proximity to the coast is weaker. Several studies have suggested that there is a relationship between the presence of pre-existing forest areas and the current distribution of disease in recent plantations around these pre-existing forests, but this hypothesis had not been directly tested as in this study (Fabre et al., 2011; Lieberei, 2007; Perkins and Matlack, 2002). The presence of A. ostoyae has also been reported in other new pine plantations in the center of France (Legrand et al., 1996). However, the origin of inoculum was not identified, because the distance between putative pre-existing forests and new plantations was not evaluated, and no historical data were available to assess the local presence of A. ostoyae before the plantations.

Two biological characteristics of A. ostoyae may have favoured the detection of the ancient land use effect on the geographical distribution of this forest disease in our study. First, A. ostoyae can spread by a clonal, underground mechanism involving the development of rhizomorphs within forest stands (Lung-Escarmant and Guyon, 2004). However, based on an analysis of genetic differentiation among several A. ostoyae disease foci, Prospero et al. (2008) suggested that the pathogen spread mostly via basidiospores at the regional scale of the Landes de Gascogne. This mode of transmission may be limited by host availability (fresh wood substrate or plants) and competitive interactions. Indeed, little is known about the efficiency of aerial dispersal of A. ostoyae to colonise new forest sites. It is assumed that the large amounts of aerial spores produced at the end of the autumn can sometimes colonise new areas by germinating on fresh wood substrates (Legrand et al., 1996). This mode of colonisation has not been observed directly in situ (Rishbeth, 1988) and it is assumed that new settlements of A. ostoyae populations would be rare and dependent on the presence of new stumps and wood fragments after recent logging events or disturbances (e.g. storms or outbreaks) during the fructification period. Because of the intensive siliculture performed for more than 100 years in the massif of Landes de Gascogne, it is likely that these favorable conditions have increased since the first plantations. However, considering the number of new observations in the massif (less than 50 each year for 1 million hectares), this suggests that the aerial colonisation is limited. Second, as A. ostoyae can also live as a saprophyte, it can survive for long periods in the soil, providing a constant source of inoculum for colonisations in the neighbourhood. The spatial aggregation of the mortality detected in this study is consistent with this mode of dispersal operating between forest stands, generating a gradient of disease from ancient sources to newly colonised areas. These two biological characteristics of A. ostoyae (a poor ability to colonise new areas and persistence in the soil after colonisation) probably explain why the pattern of expansion of the disease from the pre-existing and first planted forests is still evident 150 years after the first plantations were established. Such patterns for other pathogens, particularly for foliar pathogens, which are known to have a large capacity for dispersal (Barrès et al., 2008) which

should rapidly erase the gradient of disease from initial sources, is thus potentially more difficult to observe.

A clustered distribution of Armillaria disease was also reported on P. ponderosa in North America (Kallas et al., 2003; Lundquist, 1991). However, site-specific effects (i.e. slope, possibly associated with soil moisture and mean host height) were identified as the main causes of this geographical distribution. In the Landes de Gascogne, such factors would be expected to have only weak effects on disease prevalence. This area has a mean altitude of about 50 m, with a mean slope of about 1.25%, with an altitude gradient from 0 to 195 m running from west to east (Jolivet et al., 2007). Only slight climatic variation is observed at the scale of the massif, with relatively small variations of temperature and slightly continental tendencies in the east (Choisnel et al., 1987; Jolivet et al., 2007). Rainfall is abundant, fluctuating essentially between 800 and 1100 mm per year, with the wettest areas in the extreme south (Jolivet et al., 2007). In addition, the soils in the Landes de Gascogne are homogeneous in terms of their composition and structure. Podzols or arenosols account for about 95%of the soil, and there is no great regional variation in their distribution, except for the coastal dunes, displaying only moderate differences in pH and size particle distribution, but similar organic matter content to that of other soils in the massif (Augusto et al., 2010). Furthermore, a previous soil analysis in infected and uninfected areas of the Landes de Gascogne reported no association between soil composition and the presence of A. ostoyae (Lung Escarmant et al., 1985). We cannot entirely rule out the possibility that soil component heterogeneity over a scale of a few hundred meters can sometimes explain local differences in the frequency of reports of Armillaria disease (Termorshuizen and Arnolds, 1994), but the low level of variation observed is unlikely to explain the similarity in the geographical distribution of A. ostoyae disease observed at both the spatial scales investigated in this study. The history of the forest and plantations thus remains the most parsimonious explanation for the observed spatial distribution of A. ostoyae in the Landes de Gascogne.

Maritime pines that are less than 10 years or more than 20 years old are more likely to be affected by the disease than those between 10 and 20 years of age (Lung-Escarmant and Guyon, 2004; Robinson and Morrison, 2001). At intermediate ages, A. ostoyae can infect maritime pines, but the symptoms of the disease may be difficult to detect because they do not necessarily result in decline and mortality. This may reflect defence reactions, restricting lesion development on the roots (Robinson and Morrison, 2001). Although the observation of the disease based on a road-reporting was more difficult in the plantations of 10–25 years old which have a strong density (Samalens et al., 2007), our data were consistent with the hypothesis of an ontogenic resistance of maritime pine to A. ostoyae. In both datasets, Armillaria-related mortality was lower for trees in the 10–20 years age group than for trees from other age classes. The large-scale dataset based on reports of high mortality during more than 25 years was certainly less dependent of host ages, and it therefore confirms that the intermediate ages in maritime pines was less sensitive. However, if host age can affect the occurrence of the observations it should not affect the overall spatial distribution of disease reports in this study. Actually, the geostatistical analysis of host age in areas in which such information was available (95.5%and 27.6% for the large-scale and fine-scale datasets) did not detect any significant spatial clustering of the host age (Fig. S4).

The similarity between the two reporting campaigns in terms of spatial clustering and the observed gradient of the disease from the coast eastwards, despite differences in the density and types of mortality report, was unexpected. At the scale of the entire Landes, only high mortality rates in several areas (i.e. several tens of dead or dying trees) were reported. This may have biased the spatial pattern of disease presence obtained, with possible underestimation for areas in which A. ostoyae infects only a few trees. More systematic reporting (as for the fine-scale reporting in this study) resulted in the detection of a larger number of mortality foci (345 instead of 147), but this difference did not modify general conclusions about the directional spread of the disease from west to east or its spatial clustering. Reporting at the scale of the entire Landes area may underestimate the presence of the disease, but the areas with the largest numbers of disease severity reports would probably be correlated with the areas in which the disease was most present. The only limitation, as highlighted by the estimates of potential SRR bias, is that estimates of density in areas of low and high disease frequency are more often biased when only reports relating to large disease foci are considered.

Epidemiological studies of the current distribution of forest plantation diseases, such as the present study, are important for identifying risk factors and directing future forest management, particularly given the growing number of planted forests worldwide. For the Landes de Gascogne, the spatial pattern detected suggests that the presence of the disease is limited by the ability of A. ostoyae to disperse, with disease detected mostly in the vicinity of pre-existing forest fragments that may have served as a reservoir of inoculum. Preventive measures can be taken to reduce the gradual colonisation of this forest by A. ostoyae, by making use of poor dispersal and spatial aggregation of this fungus. For example, areas within or close to areas with a high density of Armillaria reports could be planted with less sensitive host trees, over at least one or two generations. This change in host availability might help to decrease the size of the inoculum and to limit its ability to disperse. The identification of these high-risk areas for pine plantations should also help the owners to reduce future economic losses by adapting forest management to the risks present in the area. For example, in areas with high levels of infection, the length of the rotation could be decreased to prevent a too long period of high susceptibility to A. ostoyae (i.e. in trees over the age of 20 years) and preventive measures could be taken as removing all stumps and wood fragments between each rotation. However, this study was only based on spatial correlations between current geographical distribution of the disease and locations of possible historical sources (i.e. the pre-existing forests). To confirm this spatial expansion of Armillaria disease in the massif, temporal data would be useful but likely difficult to obtain in a short term for a species growing slowly in the soil and with an apparent low success of colonisations. An alternative could be the study of the demographic history of the pathogen in the Landes de Gascogne by analysing the genetic diversity of the A. ostoyae populations. Sophisticated methods using for example approximate Bayesian computation approach, were successfully used to identify scenario of expansion in fungal pathogen species (Barrès et al., 2012; Fontaine et al., 2013). They could be used in A. ostoyae to detect a genetic signal of expansion and to estimate the intensity of the expansion in future studies.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foreco.2015.08.028.

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Supporting information

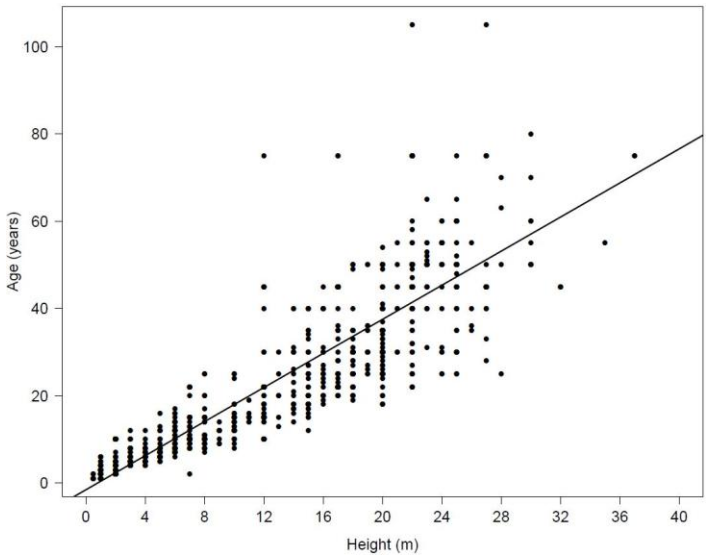

Figure S1: Relationship between the height and age of maritime pine plantations, based on the large-scale dataset. The regression line is shown in black.

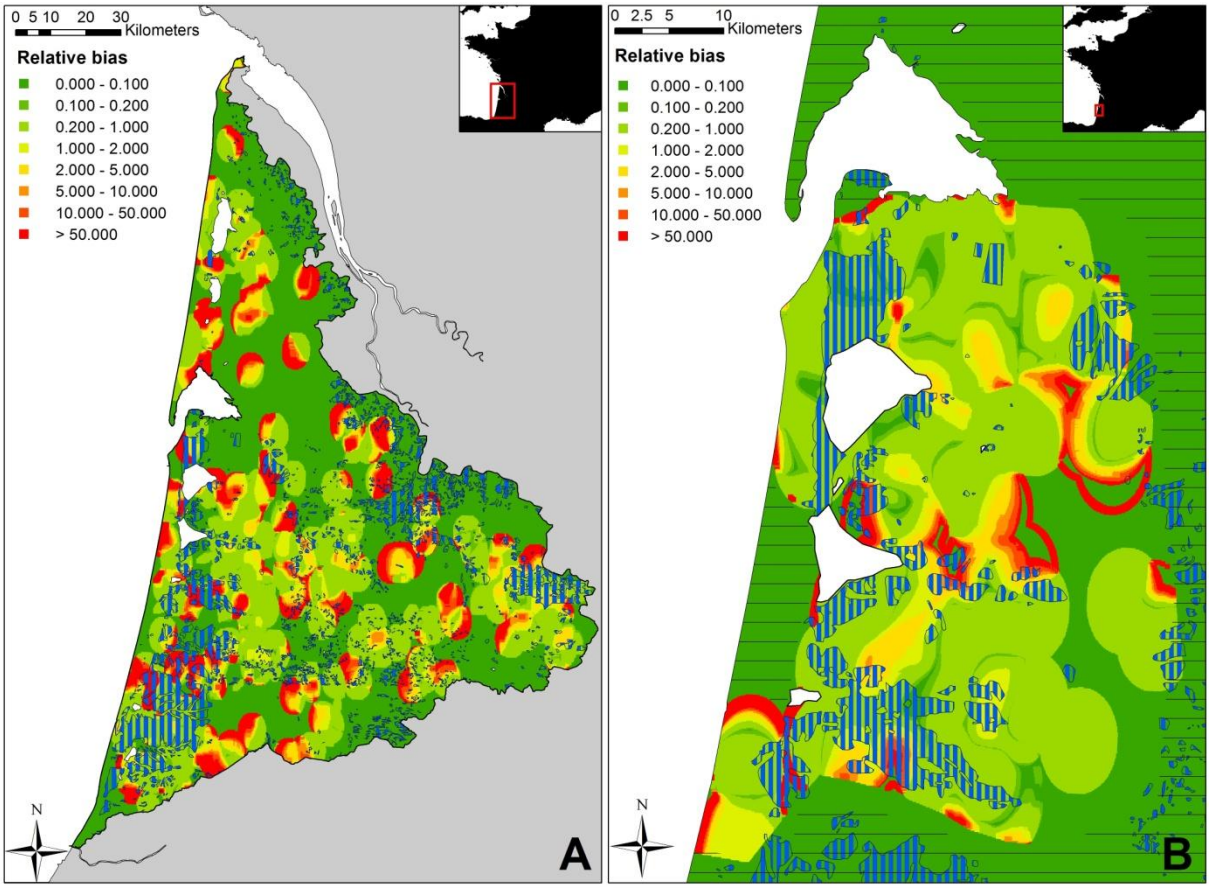

Figure S2: Distribution map of the relative potential bias in *A. ostoyae* disease reporting on maritime pine in the Landes forest of Gascogne. A: Large-scale dataset; B: fine-scale dataset. High biases are indicated in red and the lowest biases are indicated in green. The unsurveyed

areas are indicated by horizontal hatching. The pre-existing forest areas (Cassini) are indicated by blue hatched areas.

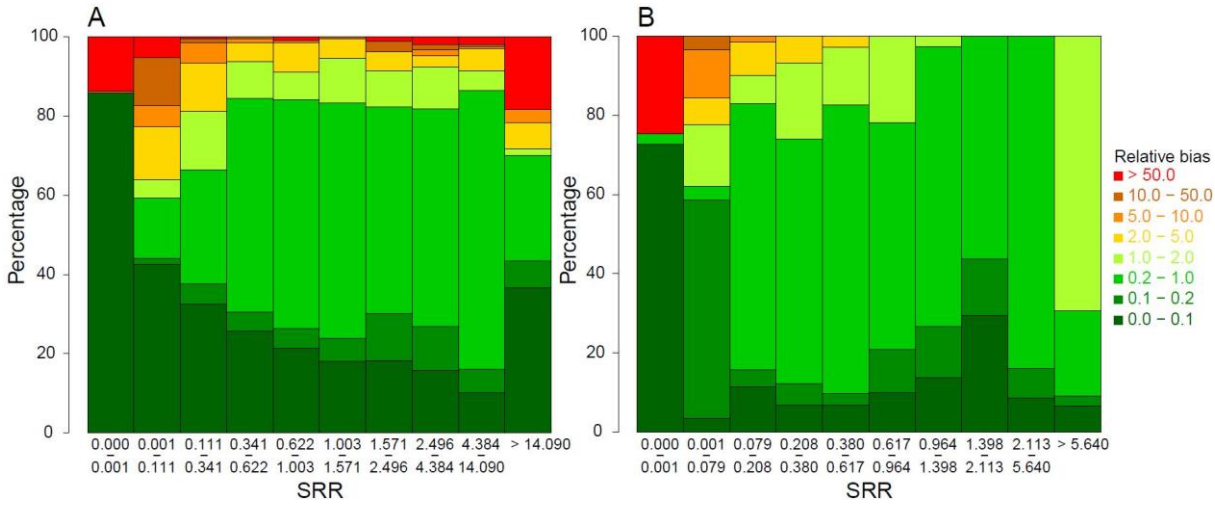

Figure S3: Proportion of relative potential bias, according to the classes of SRR. A: Large-scale dataset; B: fine-scale dataset. High potential biases are indicated in red and the lowest potential biases are indicated in dark green.

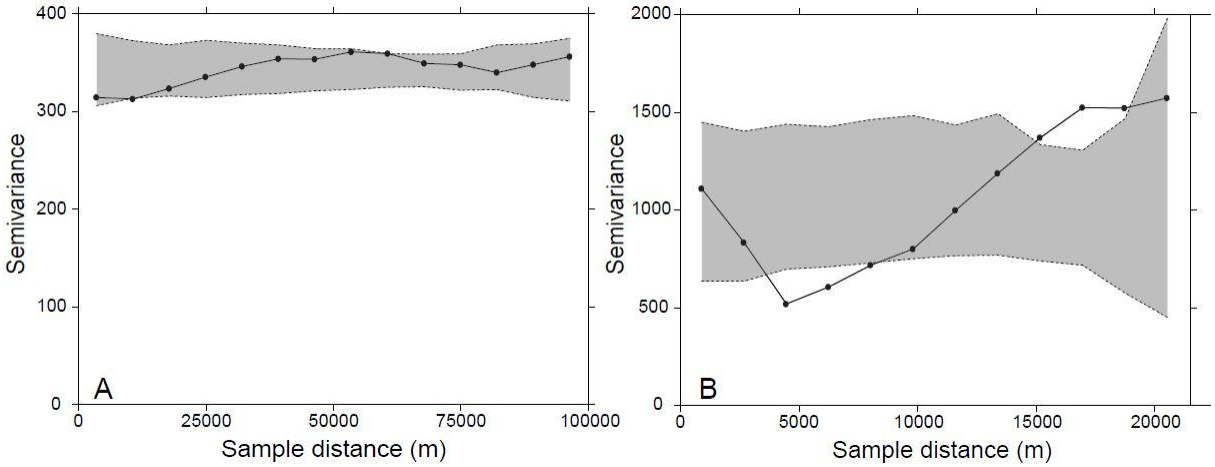

Figure S4: Semivariogram of maritime pine age. A: Large-scale dataset; B: fine-scale dataset. The grey area indicates values not significantly different from those obtained for a random structure ($P > 0.05$; obtained after 999 permutations of spatial location).

CHAPITRE III

Variation of the traits involved in the parasitism of *Armillaria ostoyae* in a maritime pine planted forest and their relationships to the saprophytism ability

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Fungal pathogen, virulence, root-rot disease, evolutionary trade-off, wood degradation

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Abstract

Armillaria ostoyae, the causative agent of root and but rot of several forest trees, has a life-cycle that alternates parasitic and saprophytic stages. It is responsible for significant mortalities in the intensively managed monospecific plantation forests of maritime pine (*Pinus pinaster*) in the Landes de Gascogne (south-western France). The objective of this study was to estimate the variation in its parasitism ability towards the maritime pine and in its saprophytism ability, and to characterize potential relationships between these traits. Experiments were carried out to assess the rate of host mortality induced by *A. ostoyae*, and two other traits involved in host infection (i.e. production of rhizomorphs and size of stem lesion). The saprophytism ability was estimated by measuring the wood-degrading capability. Results showed that the 14 studied *A. ostoyae* isolates, coming from the Landes forest, induced high mortality rates in maritime pine (77% on average), with significant variation among the isolates. Rhizomorph production was significantly correlated with virulence, suggesting that this fungal ability plays an important role in the parasitic stage of *A. ostoyae*. However, we did not detect any significant relationship between parasitism and saprophytism, components, which may suggest that there is no trade-off between these traits.

Introduction

Some wood decay fungi and *Armillaria* species, in particular, exert a strong pressure on forest ecosystems because of their life cycle which combines both parasitic and saprophytic phases on trees (Baumgartner et al. 2011; Legrand et al. 2005). Indeed, these fungi can infect living tree hosts and kill host tissues from which they feed, but they can also colonize dead wood substrates from which they draw resources. Due to these characteristics, they make contribute to carbon recycling (Baumgartner et al. 2011). *Armillaria ostoyae* (Romagn.) Herink, is a frequent root and but rot pathogen of conifers, causing significant dieback of conifers in the northern hemisphere forests (Morrison et al. 1988; Pautasso et al. 2005). This fungus exhibits different behaviors which vary from quasi exclusive saprophytism to primary parasitism (i.e. attacking healthy and unstressed trees), depending on the host and the forestry context (Guillaumin and Legrand 2005). In hardwood forests, *A. ostoyae* mainly adopts a saprophytic behavior and rarely infects living trees. In natural coniferous forests it behaves essentially as a secondary or weak parasite, infecting dominated or weakened trees (Ferguson et al. 2003; Laflamme 2010),

whereas, in intensively managed conifer plantations (e.g. thinning, short rotation period and fertilization), many studies reported that this species acts as a primary parasite (Morrison and Mallett 1996; Lung-Escarmant and Guyon 2004). In such forests, the dense and uniform host resources allow easy tree-to-tree parasite transmission, especially via clonal dispersal (Prospero et al. 2008). Different studies showed that host availability and genetic uniformity were probably the factors for an increase of disease prevalence (Gerlach et al. 1997; Korhoren 1998; Morrison et al. 1988; Pautasso et al. 2005). Under the transmission-virulence trade-off model (Anderson and May 1982; May and Anderson 1983), these environmental conditions could favor the most virulent *A. ostoyae* genotypes (Thrall and Burdon 1999), and consequently reduce the variation for this trait. However, this model is not totally adequate here. *A. ostoyae* can survive and disperse from dead hosts, and its optimal fitness may be not depending on its virulence towards living trees. Another change in *A. ostoyae* host population, caused by the sylviculture intensification, is the rapid turnover of tree populations (nowadays in average 50 years). Thus, this rapid tree rotation may favor a parasitic strategy, because the young trees, more susceptible to the parasite (Gibson 1960; Lung-Escarmant and Guyon 2004; Labbé et al. 2015), are regularly available on large surfaces for the pathogen. These short rotation periods, as well as the high number of commercial thinnings (i.e. up to 4), also contribute to increase the number of fresh stumps in the stands and thus of wood substrates which are easily colonized by *A. ostoyae*. Moreover, the intensification of forestry contributes to increase the frequency of stress in pines, especially during pruning or even as a result of damages during the mechanized clearing. Although the range of variation for the phenotypic traits associated both with the saprophytic and parasitic stages have been partially studied for some *A. ostoyae* populations (see for example Morrison and Pellow 2002; Prospero et al. 2004), it is still unknown in the special context of intensive sylviculture.

A. ostoyae infects hosts through subterranean rhizomorphs (*Rhizomorpha subterranean*). Rhizomorphs are aggregated hyphae, which expand in the soil and penetrate the woody conifer roots by enzymatic degradation and mechanical force, often in the absence of wounds (Zeller 1926; Solla et al. 2002). Infection can also occur by direct contact between root and an inoculum source, such as an infected root or a colonized woody fragment in the soil (Lung-Escarmant et al. 2003; Shaw 1980).

Once inside host tissues, the fungus develops mycelium in the host cambium. Mycelial fans form under the root bark and spread in both directions, from the root tip to the root collar. At the front of the parasite progression mycelial fans decompose the cambium and the secondary xylem by the massive production of several enzymes such as laccases (e.g. EC 1.10.3.1) and polygalacturonases which degrade lignin and pectin, respectively (Robene-Soustrade et al. 1998). Other proteins involved in pathogen protection against the host defense, such as peroxydases, and in toxin production, might facilitate the infection, as shown for example in *Heterobasidion annosum* another root-rot fungus (Olson et al. 2012). Although several homologs of these genes have been described (Ross-Davis et al. 2013), their effect in the success of infections remains largely unknown in *A. ostoyae*. Its virulence, defined in our study as its ability to induce host death, likely results of the additive effects of these two first stages: the rhizomorph production (the contact and penetration stage) and the ability to cause lesion in the cambium of the hosts (the necrotrophic stage). At our knowledge, although the effect of the pathogen on the different plant tissues has been largely described (Solla et al. 2002; Cleary et al. 2012), the variation in the necrotrophic activity production has not been studied yet.

After the host death, *A. ostoyae* having a saprotrophic wood-degrading ability, behaves as white-rot fungus (the saprophytic stage). Throughout the dead wood, the fungus forms black zone lines (pseudosclerotial plates), which consist of pigmented hyphae located in the tracheids and delimiting the infected wood (Campbell 1934; Lopez-Real 1975). Robene-Soustrade et al. (1998) showed that enzymes involve in wood-degrading ability of the *Armillaria* species are different from those involved during the necrotrophic stage. The Mn-peroxydase, which targets the lignin, is produced in the first stage of wood degradation process, whereas the CM-cellulase and the xylanase, respectively involved in the cellulose and the hemicellulose degradation, are produced during the later stages of the wood degradation process. Although the wood-degrading ability of *A. ostoyae* is low in comparison to other wood-decomposing fungi (Guillaumin and Lung 1985; Robene-Soustrade 1993; Prospero et al. 2004), the fungus can persist in the stumps during more than 40 years (Rishbeth 1972). This ability to stay in the buried wood could be the result of an important adaptive process in forest ecosystems where host species and susceptible age classes are not always present at the good time and the good place (Smith et al. 1992; Baumgartner and Rizzo 2002; Ferguson et al. 2003). If an evolutionary trade-off exists between

saprophytism and parasitism stages in planted forests where hosts are nearly always present in large density, individuals exhibiting strong wood-degrading ability might be counter-selected. Then this phenotypic trait should have a low mean value and a small variance in the *A. ostoyae* population.

In the Landes de Gascogne forest, one of the largest mono-specific pine plantation in Europe (approximately 1 million ha) located in the south-western France, *A. ostoyae* has been first described in 1920 (Guyot 1928), and then increasingly reported on maritime pine (*Pinus pinaster*) for 30 years (Aumonier 2007; Lévy and Lung-Escarmant 1998). This disease emergence is associated with a change in forest sylviculture, which has been initiated when maritime pines were intensively planted (after the 1857 law) and accelerated after the Second World War (Temple 2011). How may this change in land use be associated with a change in the life history traits of *A. ostoyae*? Our knowledge in genetic variation in *A. ostoyae* traits, especially for populations in managed pine forests, is still insufficient to answer this question and to predict evolutionary dynamic of *A. ostoyae* in planted forests. However, it could be assumed that the strong virulence variability of the pathogen and the intensification of the forestry in the Landes de Gascogne, allowed the selection of the most virulent isolates which could quickly colonize the forest. Our first objective was thus to assess the variation in *A. ostoyae* virulence for maritime pine and to test the relationships with other biological traits likely involved in the parasitism of this species. Therefore, we assessed the rhizomorph production and the virulence of 14 *A. ostoyae* isolates by experiments targeting each of these traits independently. According to Prospero et al. (2004) and Omdal et al. (1995), rhizomorph production is positively correlated with induced mortality on young seedlings tested in experimental conditions. However, some observations question the value of this correlation in the maritime pines of the Landes de Gascogne. First, rhizomorphs are rarely observed *in situ* in this forest where dry and sandy soils are not favorable for their production (Guillaumin and Legrand 2005). Consequently, root contacts and ability to rapidly invade root tissues would be more important to predict *A. ostoyae* induced mortality in such conditions than rhizomorph production. Second, in the two studies previously cited, these two traits were not independently tested, since rhizomorph production was estimated from the infection trial, with the inoculum located at several centimeters from seedlings (see for example Prospero et al. (2004). Under such experimental conditions, the speed of which the fungus reaches its host throughout the soil is

probably better estimated than the ability to rapidly colonize the host tissues (see below). Thus, the involvement of rhizomorph production in virulence of *A. ostoyae* in such environmental context remains to be investigated more precisely. Moreover, we studied the lesion formation ability of the pathogen, which is assumed to have an important role in the parasitic phase (Baumgartner et al. 2011). The *in vitro* growth rate of each isolates was also measured assuming that growth on "medium culture" may be a correct proxy of the fungus growth *in planta* and sometimes well correlated with fitness in filamentous fungal species (Pringle and Taylor 2002). Our second objective was to assess the relationships between parasitic and saprophytic ability of the pathogen, and to test the existence of a trade-off between these two components of life-cycle in the context of the maritime pine forest of the Landes de Gascogne. Prospero et al. (2004) showed that *A. ostoyae* isolates from Norway spruce forests of Switzerland presented no trade-off between the two phases of its life-cycle. However, Swiss forests are not characterized by intensive plantations as in the south-western France, and *A. ostoyae* develops in the heartwood of spruce that possibly decreases the trade-off between the necrotrophic and the saprophytic phase. We hypothesized that such a trade-off might induce *A. ostoyae* isolates investing in virulence less efficient in saprophytism and vice versa.

Materials and methods

1. Host plants

Plant root inoculations were conducted on 2-year-old maritime pine seedlings. We used plants for a half from unimproved provenances, which consisted of a mixture of seeds collected from several stands of maritime pine located in the Landes de Gascogne forest, and for the other half, from one improved variety of maritime pine (VF2-Saint-Augustin). These seeds were collected in the seed orchard of the INRA (French National Institute for Agricultural Research) breeding program focused on growth rate and stem straightness (Bouffier et al. 2008). At 1-year-old, each bare-root seedling was planted in a 5.5 l plastic cylindrical pot (20 **x** 25.7 cm) containing wood fibre (50%), blond peat (30%), mineral soil (20%) and fertilized with 6 g l^{-1} of slow-releasing fertilizer (Osmocote Exact Lo.Start).

2. *Armillaria ostoyae* isolates

Fourteen *A. ostoyae* isolates (A1-14), sampled on dead maritime pines randomly distributed at the west of the Landes de Gascogne forest were studied (Labbé et al. 2015). All isolates were sampled in 2012, except two (A3 and A4, sampled in 2008 and 2010 respectively). Infected wood

samples, collected at the collar in zones colonized by characteristic mycelial fans of this pathogen, were cut into pieces of about 5 mm long and were surface sterilised during 8 min in sodium hypochlorite (3 % active chlorine). After being dried with paper towels, they were placed on Petri dishes (90 mm in diameter) containing MAT medium (20 g l^{-1} of malt extract, 15 g l^{-1} of Bacto Agar, 100 mg l^{-1} of penicilline, 100 mg l^{-1} of streptomycin and 0.1% of thiabendazole) (Guillaumin 1977). Petri dishes were incubated at 25°C in the dark during approximately four weeks and were then transferred to Petri dishes containing 2 % malt agar medium (20 g l^{-1} of malt extract and 15 g l^{-1} of Bacto Agar). After four weeks at 25°C, Petri dishes were stored in collection at 4°C in the dark. Genotyping at five microsatellites loci (Langrell et al. 2001; Prospero et al. 2008), showed that each isolate represents a different *A. ostoyae* genotype (data not shown). *In vitro* growth was estimated on twenty replicates for each isolate cultivated on 2 % malt agar medium, after a 28-day incubation period at 25°C in the dark (Rishbeth 1986), and using the software ImageJ v.1.46 (Rasband 1997).

Inoculums were prepared as described by Guillaumin and Lung (1985): 20 sticks (7.5 cm long, 1.5 cm diameter) of hazel (*Corylus avellana L.*) were put into 750 ml glass containers and filled with osmosed water. The containers were thereafter autoclaved twice (20 min, 121 °C and 1.1 bar) with osmosed water replaced between each. Osmosed water was then removed and replaced by a nutrient medium (½ l of osmosed water + ½ l of Knorr® vegetable soup + 10 g l^{-1} of Bacto Agar) and the containers were autoclaved again. Finally, in each container 4 plugs (7 mm diameter) of a *A. ostoyae* culture were put on the top of four hazel sticks. Containers were stored before use at 24 °C in the dark during 3 months.

3. Plant roots inoculation

To inoculate *A. ostoyae* in pine seedlings, our objective was to infect roots via the rhizomorphs initiated from an inoculum source, simulating closely the natural conditions prevailing for infection. The inoculum sticks were carefully put into each plant pot at two centimeter of the root system. Each isolate was inoculated in 18 pines plants of the improved maritime pine variety and 18 of the unimproved maritime pine provenances. For the control modality, non-infected hazel sticks were used. Overall, 504 pine trees were inoculated on May 2013. All plants were installed, according to a complete randomized experimental design, in frost-free greenhouse, and watered every two days. The two first monitorings of pine mortality occurred at one and two months after the inoculation, then the pine mortality was evaluated every two weeks and during six month after inoculation.

Only dead plants, in which subcortical mycelial fans were observed at the collar, were diagnosed as killed by *A. ostoyae*. At the end of the experiment, the inoculum stick viability of each surviving plant was assessed by checking the presence of viable mycelial fans under the bark of the inoculum stick.

4. Cut stems inoculation

Fifteen branches (about 50 cm long, 1 cm diameter) were cut from ten maritime pines (from the improved variety previously described) of 10-year-old. Each *A. ostoyae* isolate (plus a mock modality) was inoculated on each tree at least once. An inoculum stick of hazel (1 cm long, 0.5 cm diameter) was placed directly into contact with the cambium on a wound (circular area of 0.5 cm diameter). The inoculum was kept in place with stretchable plastic film, wrapped with aluminum foil to avoid light. Control treatments consisted of wounded stems without inoculum. The stem diameter was measured at the inoculation site. After a three-week incubation, during which the pine segments were kept alive in water, the stems were debarked around the inoculation sites. The outer edges of the lesions were traced onto tracing paper and scanned on an Epson Perfection 2400 dpi photo scanner. The area of each lesion was then calculated with ImagJ v.1.46 software.

5. Rhizomorph production

The rhizomorph production of each *A. ostoyae* isolate was quantified by their biomass. Hazel sticks (see above) were inoculated to serve as growth substrate for each isolate (15 segments per isolate). They were put at the middle of 1.5 l plastic cubic pots (13 x 13 x 13 cm) filled with sand (Redfern 1973). These pots were placed, according to a complete randomized design, in climatic chamber at 21°C and were humidified three times per week with 100 ml of water in order to maintain a high level of moisture content. After nine months, the number and total dry weight of rhizomorphs, which were formed on each hazel sticks, were estimated (Morrison 2004; Prospero et al. 2004). When no rhizomorph was observed on a stick, the presence of viable mycelial fans under the bark of these sticks, attesting the success of stick colonization by *A. ostoyae*, was checked.

6. Wood-degrading ability

The wood-degrading ability was assessed on the norm AFNOR NF X 41-502 (Delatour and Sylvestre-Guinot 1978), and was adapted according to Robene-Soustrade (1993). Stem segments of maritime pine without bark (0.5 x 1 x 3 cm) were dried (60 °C for 96 h) and weighed once and then autoclaved (3 h, 105 °C and 1.1 bar) before being rewetted 10 min in sterile water. Each segment was then placed in the center of a Petri dish

containing 15 ml of 2 % malt agar. Two small transplants (7 mm in diameter), coming from the margin of *A. ostoyae* colonies growing on 2 % malt agar medium, were then placed on either side of the maritime pine segment. The Petri dishes were placed in an incubator (24 °C and 85% humidity) during 6 months after which the pieces of wood were cleaned of the mycelium, dried and weighed. The percentage of mass loss of the pine segment was used to evaluate the saprophytic ability of the tested isolates. Fourteen replicates were performed for each of the 14 isolates, as well as 14 controls, consisting in stem segments but without any inoculum.

7. Statistical analysis

All statistical analyses were perform in R 2.15.1 statistics software (R Core Team 2012). To compare virulence of *A. ostoyae* isolates and pine susceptibility we used the Kaplan-Meier estimate of survival pine probabilities using the log-rank test (Collett 2003) and performed with coin package (Zeileis et al. 2008) available in R. In order to test for significant differences in lesion size among isolates, we performed analysis using linear mixed effect (LME) model with the nlme package of R (Pinheiro et al. 2011). The isolate and the stem diameter at inoculation sites were considered as fixed effect in this model with host tree as a random effect. The *in vitro* growth rate, the rhizomorph weight, the number of rhizomorph initiations and the wood-degrading ability, which were normally distributed, were compared with ANOVA and Tukey's test. The relationships between the induced mortality, the rhizomorph production, the lesion formation ability, the wood-degrading ability and the *in vitro* growth rate of each isolates were examined with linear regression and by means of Pearson correlation coefficient determined with R and displayed with the psych package of R (Revelle 2015).

Results

1. *Armillaria* induced mortality

All inoculum sticks of survival trees at the end of the experiment contained viable mycelial fans. Out of the 504 pine plants inoculated with the pathogen, 22 plants (4%) died with no observation of lesion and mycelium, suggesting a death independent of *A. ostoyae* infection. Similarly, eight of the 35 control plants (23%) have also died during the experiment for unknown causes. These mortalities, not induced by *A. ostoyae*, were excluded from analyses. First *A. ostoyae* induced mortalities were observed for all isolates 70 days after inoculation, with rates varying from 3 to 61% (Fig. 1). Two isolates, A1 and A14, were the most virulent, and caused more than 60% of mortality after 70 days. At 120 days,

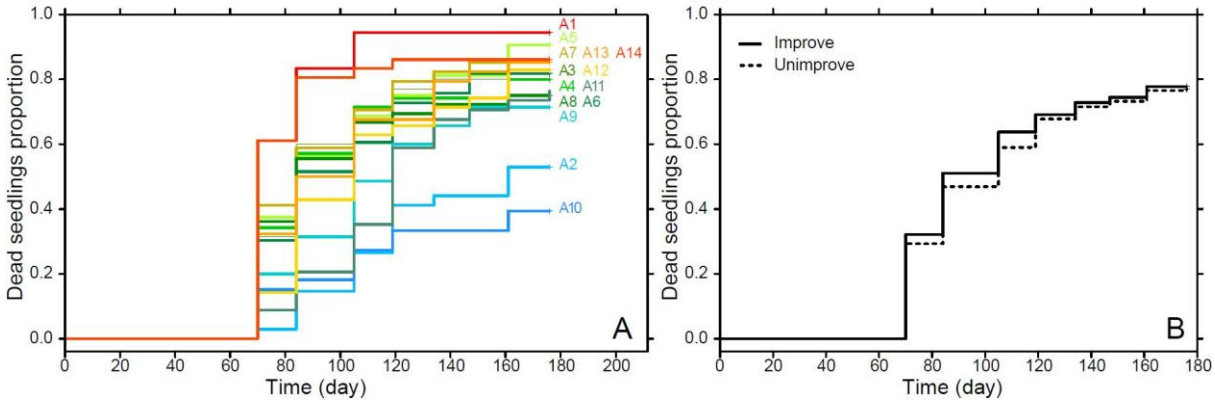

Figure 1: Plot of mortality probabilities (1 - survival probabilities), using the Kaplan-Meier estimate, of *P. pinaster* inoculated with 14 different *A. ostoyae* isolates: A: according to isolates (each different color line corresponds to a tested isolates); B: according to maritime pine provenances (each line type corresponds to a provenance). X axis: days from the beginning of inoculation; Y axis: proportion of dead seedlings.

Armillaria induced mortality was superior to 50% for all isolates, except two (A2 and A10, 41% and 33% of mortality respectively). Except for few rare exceptions (A5), isolates ranking at 120 days was the same as at 180 days (Wilcoxon rank test, Value = 27, *P*-value = 1). At the end of the experience (180 days), the mean mortality rate was 0.77, and the minimum and the maximum were respectively 0.39 (A10) and 0.94 (A1). According to the survival Kaplan-Meier analyses, the host origin did not influence the global mortality curves (log-rank test, Value = 2.52, *P*-value = 0.28; Fig. 1B), neither the mortality induced by each isolate, except for A4 which showed higher mortality rate for unimproved maritime pines (log-rank test, Value = -2.10, *P*-value = 0.04; Fig. S1). The survival analyses also showed a significant variation in induced mortality among *A. ostoyae* isolates (log-rank test, Value = 74.87, *P*-value < 0.001).

2. Necrotrophic ability

Because one isolate failed to develop on the inoculum sticks (A2), only 13 of the 14 isolates were studied. After a three-week incubation, the wounding had caused a discoloration (surface of 0.02 cm² ±0.02 on average) visible on control stems around the circular wounding area. By comparison, inoculation by *A. ostoyae* isolates caused under-bark lesions, which surface varied from 0.42 cm² (±0.22; A14) to 3.28 cm² (±1.58; A12), with a mean of 1.10 cm² (±0.22). The average maximum length of the lesions (i.e. from the inoculum site to the edge of the lesion) was 1.51 cm (±0.19) and ranged from 0.70 cm (±0.21; A8) to 2.40 cm (±0.74; A12). No significant isolate effect could be detected

Test	Variables	Source of variance	Df	*F*-value	*P*-value
ANOVA	Rhizomorph weight (mg)	Isolate	13	8.97	< 0.001
	Number of rhizomorph	Isolate	13	24.41	< 0.001
	Wood-degrading ability (%)	Isolate	13	13.77	< 0.001
	in vitro growth (cm²/day)	Isolate	13	40.92	< 0.001
LME	Necrotrophic ability	Isolate	12	1.48	0.15
		Stem diameter (mm)	1	0.30	0.58
		Isolate * Stem diameter	12	1.10	0.37

Table 1: Results of the ANOVA and LME for analysis of the rhizomorph production, the wood-degrading ability, the *in vitro* growth rate and necrotrophic ability of the 14 *A. ostoyae* isolates. Rhizomorph production corresponds to the weight (mg) and the number of rhizomorph initiated from the inoculum stick after nine months. The saprophytic ability was notified by the percentage of weight loss of maritime pine fragments inoculated during six months. The *in vitro* growth rate corresponds to the mean mycelial produced in culture per day (cm²/day). Finally, the necrotrophic ability was expressed as the average size of the lesion (cm²). Degrees of freedom (Df), *F*-value and *P*-value are shown.

(*P*-value = 0.15; Table 1). The stem diameter and its interaction with the isolate had no significant effect on the size of the lesion surfaces (Table 1).

3. Rhizomorph production

The mean of total dry weight of rhizomorphs produced after 9 months was 41.0 mg (±10.7), and varied from 0.5 mg (±0.3; A10) to 148.8 mg (±32.5) for the most productive isolate (A4). The number of rhizomorph initiations ranged from 0.2 (±0.1; A13) to 41.5 (±3.9; A9), with a mean of 12.6 (±1.9). There was a significant effect of isolate on rhizomorph production and on rhizomorph initiations (*P*-value < 0.001 in both cases; Table 1; Fig. 2). These two components were positively correlated (Pearson correlation test, r = 0.14, *P*-value = 0.04).

4. Wood-degrading ability

The mean weight loss of stem segments of maritime pine, after a six-month incubation with the different *A. ostoyae* isolates, was 7.57% (±0.86) and varied between about 3.47% (±0.57; A7) and 14.71% (±1.39; A4). A significant effect of isolate was detected (Anova, *F*-value = 13.77, *P*-value < 0.001; Table 1).

5. Relationships between *A. ostoyae* traits

According to the positive correlation between the two components of the

rhizomorph production (i.e. number and weight of rhizomorph), rhizomorphs weight, which was more integrative, was used to study correlations of rhizomorph production with other traits. We assessed correlations with virulence by considering mortality at 120 and 180 days after inoculation. However, we presented only results for virulence at 120 days, except when some differences were observed. The virulence was significantly positively correlated with the rhizomorph production (r = 0.61, *P*-value = 0.02; Fig. 2C). However, 180 days after inoculation, this correlation was no more significant (r = 0.42, *P*-value = 0.13). The necrotrophic ability was also positively, but not significantly, correlated with the virulence (r = 0.17, *P*-value = 0.58 respectively; Fig. 2D), and no relationship was observed between this virulence component and the rhizomorph production (r = 0.08, *P*-value = 0.79; Fig. 2G). The ability to grow in culture, which exhibited significant differences after 28 days in culture (*P*-value < 0.001), with rates ranging from 0.22 cm² per day (±0.02; A8) to 0.67 cm² per day (±0.01; A4; Table 1), was not associated with the virulence (r = 0.02, *P*-value = 0.94) or with one of the other two components of virulence tested (r = 0.41, *P*-value = 0.15 and r = 0.27, *P*-value = 0.38, for the rhizomorph production and the necrotrophic ability, respectively; Fig. 2B I and F, respectively). In contrast, isolates with the fastest *in vitro* growth were also those with the highest ability to decompose dead wood (r = 0.73, *P*-value = 0.003; Fig. 2J). The saprophytic ability was not correlated with the virulence (r = 0.05, *P*-value = 0.86), neither with the two main components of virulence analyzed in this study, i.e. the rhizomorph production (r = 0.31, *P*-value = 0.28) and the lesion formation ability (r = 0.09, *P*-value = 0.78; Fig. 2A, H and E, respectively). The exclusion of the isolates not sampled in 2012 did not change the relationships described above (Fig. S3), except for the relations established between the rhizomorph production and the growth *in vitro* as well as with the wood-degrading ability, which became slightly negative although still not significant (r = -0.07, *P*-value = 0.83 and r = -0.21, *P*-value = 0.52 respectively; Fig. S3I and H).

Discussion

A significant variation of virulence among the 14 isolates was detected in this study. Similar significant variations in virulence between different European isolates of *A. ostoyae* were also observed on other host species (Gregory 1985; Morrison 2004; Prospero et al. 2004; Rishbeth 1982). Interestingly enough, some studied isolates showed a mild virulence for maritime pine seedlings. This seems to be contradictory with the hypothesis of increasing virulence with

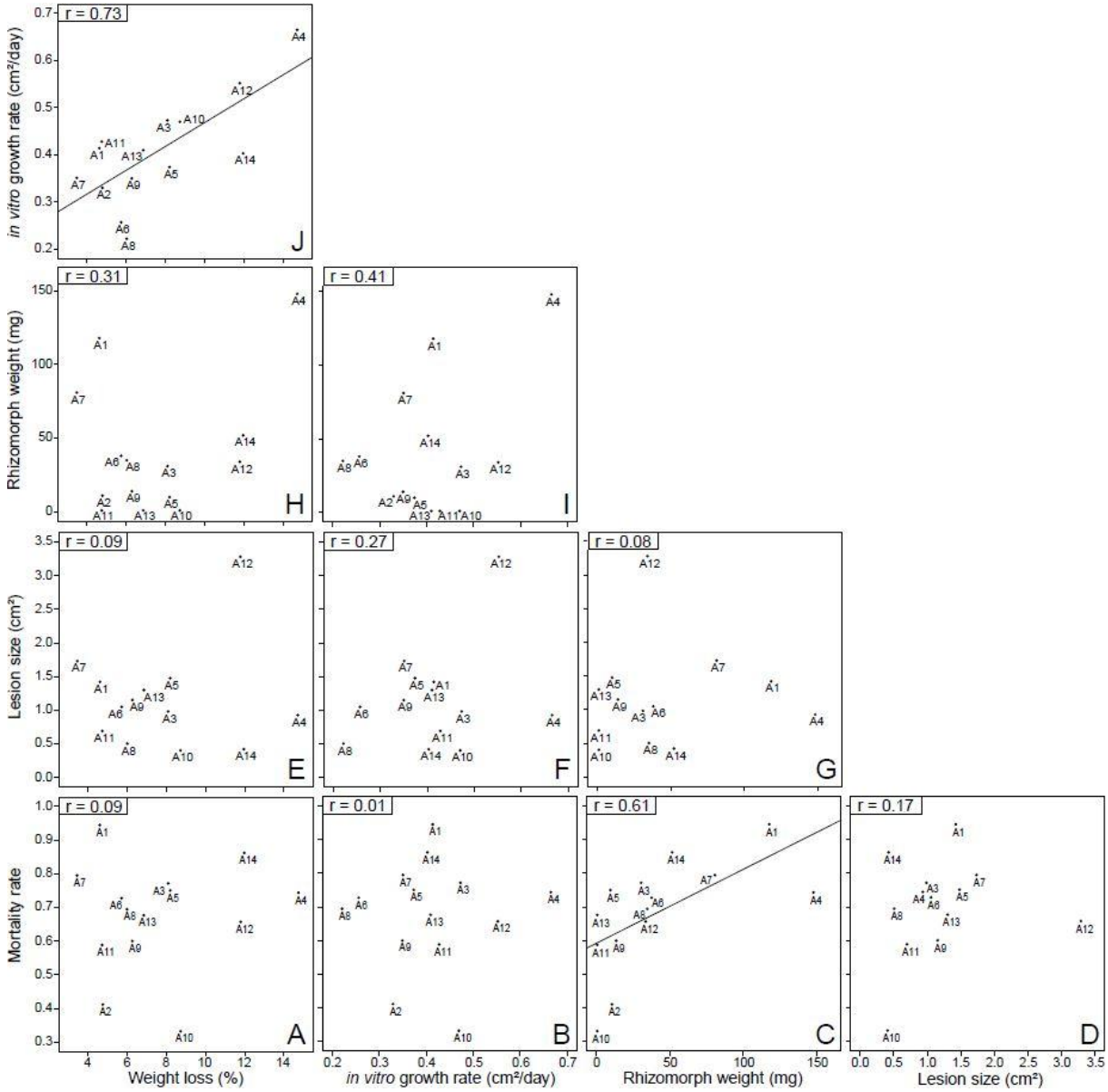

Figure 2: Relationships between the virulence, the rhizomorph production, the *in vitro* growth rate, the necrotrophic and the wood-degrading ability of each 14 *A. ostoyae* isolates. The black lines represent significant linear regressions. Each Pearson correlation coefficient (r) is indicated in the corner of each plot.

increasing forest management and a reduced variation due to the selection pressure. Although all studied *A. ostoyae* isolates were removed during the parasitic phase and on dead trees, it cannot be excluded that some of them behaved as secondary parasites and were found in trees which died from another biotic or abiotic factors (e.g. climate, insects and other diseases). These mild parasites could have outcompeted more virulent isolates due to a priority effect. Isolates collected from stumps or buried stem segments should be more systematically looked for, to compare their virulence with those collected on a recently infected tree. The mild virulence can also be consistent with our hypothesis if we consider that most of

the forests, in the Landes de Gascogne, is not older than 150 years, and thus adaptive processes are probably still in progress. Another explanation may reside in the precise origin of the studied isolates. Indeed pathogens may be more virulent at the front of their dispersal range spread when they spread in a naive host population (Phillips and Puschendorf 2013). Thus *A. ostoyae* isolates collected in the ancient pathogen distribution area might be less virulent than those collected in the area of new disease expansion. The number of isolates used in this study was too low to carry out a real test of this hypothesis. However, it could be envisaged in future studies to compare the virulence of isolates collected within pre-existing forests with isolates collected within newly planted forests since the second half of the 19th century, since previous inventories and epidemiological studies suggested *A. ostoyae* origin in the pre-existing forests (Lévy and Lung-Escarmant 1998; Aumonier 2007; Labbé et al. 2015).

Although our experimental conditions were slightly different from the studies of Prospero et al. (2004) and Omdal et al. (1995), our results suggest that maritime pines of Landes provenances, were more susceptible to *A. ostoyae* (77% of mortality after 6 months) than Norway spruce (about 19% after 30 months) or other tree species (e.g. after 30 months: 8% on white fir, 32% on lodgepole pine; Omdal et al. 1995). Alternatively, *A. ostoyae* isolates from the Landes de Gascogne forest could have a higher virulence than those from other forests, because of a strong host adaptation induced by intensified monoculture. However, preliminary unpublished study did not indicate that south-western French isolates were more virulent on maritime pine than Swiss isolates collected on Norway spruce (F. Labbé, unpublished results), suggesting that host adaptation is low or inexistent. Improved varieties of pines, selected for their growth and for their straightness, might also have favored the increase of *A. ostoyae* susceptibilty of these pines. Although intraspecific variability of resistance to *A. ostoyae* has been reported among maritime pine provenances (Lung-Escarmant and Taris 1988), we did not observe, in average, any difference in induced mortality between improved and not improved pine plants. This result could suggest that breeding for better growth was not done in detriment of disease resistance in maritime pine. Finally, estimate of virulence is not representative of virulence in the field, as shown for example with the white fir in North America (Omdal et al. 1995). Most of the time in the field, the pathogen must infect older age classes than seedlings of 2 years-old. For older ages, this variation in virulence may be lower or higher, and representative of the real optimum between virulence and transmission. Unluckily, it is difficult to inoculated old trees in

controlled conditions, and thus the disease resistance assessment should be carried out not only in greenhouse but also in the field, and with plants at different age classes and with more precise resistance assessments.

We also estimated significant differences in rhizomorph production among isolates that have already been reported in previous studies (see for example Morrison 2004; Prospero et al. 2004). In Morrison (2004), inoculum sticks placed four months in plastic bags, which containing soil, produced from up to six times more rhizomorphs (0.28 g) than in our experiment lasting nine months. By contrast, only one to four rhizomorphs were initiated after 30 months in Prospero et al. (2004), which was much less than we observed. These differences could highlight firstly the large variation in rhizomorph production observed among isolates of *A. ostoyae*, but could also result from the different methodologies used in these studies. Indeed, according to the significant effect of greater moisture content on the rhizomorph growth (Pearce and Malajczuk 1990), the moisture conditions generated inside the plastic bags used by Morrison (2004) were potentially favorable for the rhizomorph production by *A. ostoyae*. Thus, as it appears to be crucial, as proposed by Aguilar-Trigueros et al. (2015), to standardize and share methods of identification and description of fungal traits in order to better compare fungal populations and understand their evolution under different environmental conditions.

Our results showed a positive correlation between induced mortality and the rhizomorph production in agreement with Prospero et al. (2004). This correlation was significant only at 120 days, which suggests that pine infection through rhizomorphs is dependent on rhizomorph production and that isolates with the more efficient rhizomorph production are the earliest ones to infect and kill pines. However once pines are infected, the induced mortality rate is likely dependent on other factors linked to pine root colonization. Although the distance of inoculum to the host was short in our experiment, the first stage of infection associated with the rhizomorph production was still determinant. The speed of initiation of rhizomorph and production of efficient molecules to penetrate the bark could be associated with the rhizomorph growth. The decrease of correlation becoming not significant after 180 days, could be due to the minimal time to carry out this first stage of life-cycle for most of the isolates. It suggests that virulence assessment should be performed in our experimental conditions, four months after inoculation in order to optimize discrimination of isolates.

Unluckily, a significant phenotypic variation for the necrotrophic stage among

isolates (i.e. the ability to colonize the host tissue) was not observed. A high variation in sizes of lesions induced by *A. ostoyae* was observed within isolates, but was not significant among isolates. This high within isolate variation may be due to a too limited number of repeats for each isolate (Figure S2). It is worth noting that the estimate of surface or length of the necrosis is difficult to estimate because of the incertitude of the limit of the necrosis visually determined. Other factors can create artifactual signal of necrosis (e.g. brown tissues in the green cambial area) as such ancient wounds, attacks of insect (e.g. *Ips sexdentatus*), or irregular thickness of the bark. Second, physiological status of the cut stem could strongly affect the plant response to infection and more repeats are required to reduce this random effect. Because it is not possible to increase much higher the number of stems, thus we need to develop or use other methods. For example, the use of the recently designed taxon-specific primers developed by Gonthier et al. (2015) for the detection and the identification several wood decay fungi of conifer including *Armillaria* spp., could allow a quick and simple method to detect low presence of *A. ostoyae* in the stem. Such a methodology to estimate the necrotrophic abilities of the isolates is absolutely needed to understand the factors explaining the observed virulence. For the moment it is impossible to conclude whether only the rhizomorph production contribute to this virulence or it is a combination of this first stage of infection and the development in cambial and wood tissue of host.

A. ostoyae also exhibited significant variation in saprophytic ability. We observed, despite some differences in the protocol, similar results than those of Prospero et al. (2004) and Robene-Soustrade (1993) reporting an average weight loss of stem segments of 22.4% after 1 year and of 11.66% after 6 month respectively, higher but consistent with the 7.57% average weight loss measured after 6 months in our study. This wood-degrading ability of *A. ostoyae* was relatively low compared to some white-rot fungi, for which the mass loss percentages can exceed 20% in just 80 days and may even reach up to 40% for *Pycnoporus coccineus* and to 50% for *Corolius hirsutus* over the same period of time (Tanesaka et al. 1993). These results confirm that, among wood-decomposing fungi, *A. ostoyae* species is not a major competitor for the dead wood degradation. This low wood-degradation ability is however sufficient enough to allow the pathogen to remain a long time in the colonized wood, and to allow the infection of a new living root growing at proximity. In agreement with Prospero et al. (2004), we did not find any significant correlation between the saprophytic ability and virulence or other associated traits (necrosis or rhizomorph

production). Therefore, although involving different enzyme complexes, no trade-off between parasitism and saprophytism would occur in *A. ostoyae*. Nevertheless, as mentioned earlier all tested isolates were collected from dead maritime pines which could potentially bias partially the relationships between these two traits. Additional studies must be performed to more firmly conclude. On the other hand, *in vitro* growth rate was positively and significantly correlated with the ability of the pathogen to degrade dead wood. This measure would be a simple, rapid and effective method for indirectly estimating the saprophytic capacity of *A. ostoyae* isolates.

Due to the apparent absence of a parasitism and saprophytism trade-off, it seems that there is no evolutionary constraint against the increase in the virulence of *A. ostoyae*, except if the dispersion is opposite to the parasitism. Indeed, *A. ostoyae* can spread through the air with the production and wind dispersal of spores during the fruiting season in the fall (Guillaumin et al. 1993). Although different studies indirectly reflect its implication in the establishment of new foci of the disease (Rishbeth 1988; Legrand et al. 1996; Prospero et al. 2008; Dutech et al. 2011), no study allow to test and compare the ability of *Armillaria* isolates to disperse over long distances. However, this could be estimated indirectly and easily in future experiment through *in vitro* fruiting abilities of *A. ostoyae* isolates (Korhonen 1980; Guillaumin 1986).

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Supporting information

Isolate	Longitude	Latitude	Year of isolation
A1	-1.043	44.594	2012
A2	-1.012	44.534	2012
A3	-1.203	44.510	2008
A4	-1.186	44.531	2010
A5	-0.976	44.233	2012
A6	-0.992	44.272	2012
A7	-1.066	44.265	2012
A8	-0.944	44.587	2012
A9	-0.972	44.568	2012
A10	-1.041	44.285	2012
A11	-1.158	44.303	2012
A12	-1.048	44.235	2012
A13	-1.176	44.251	2012
A14	-1.172	44.256	2012

Table S1: Origin of the 14 *A. ostoyae* isolates used in virulence and saprophytic ability assessment experiments.

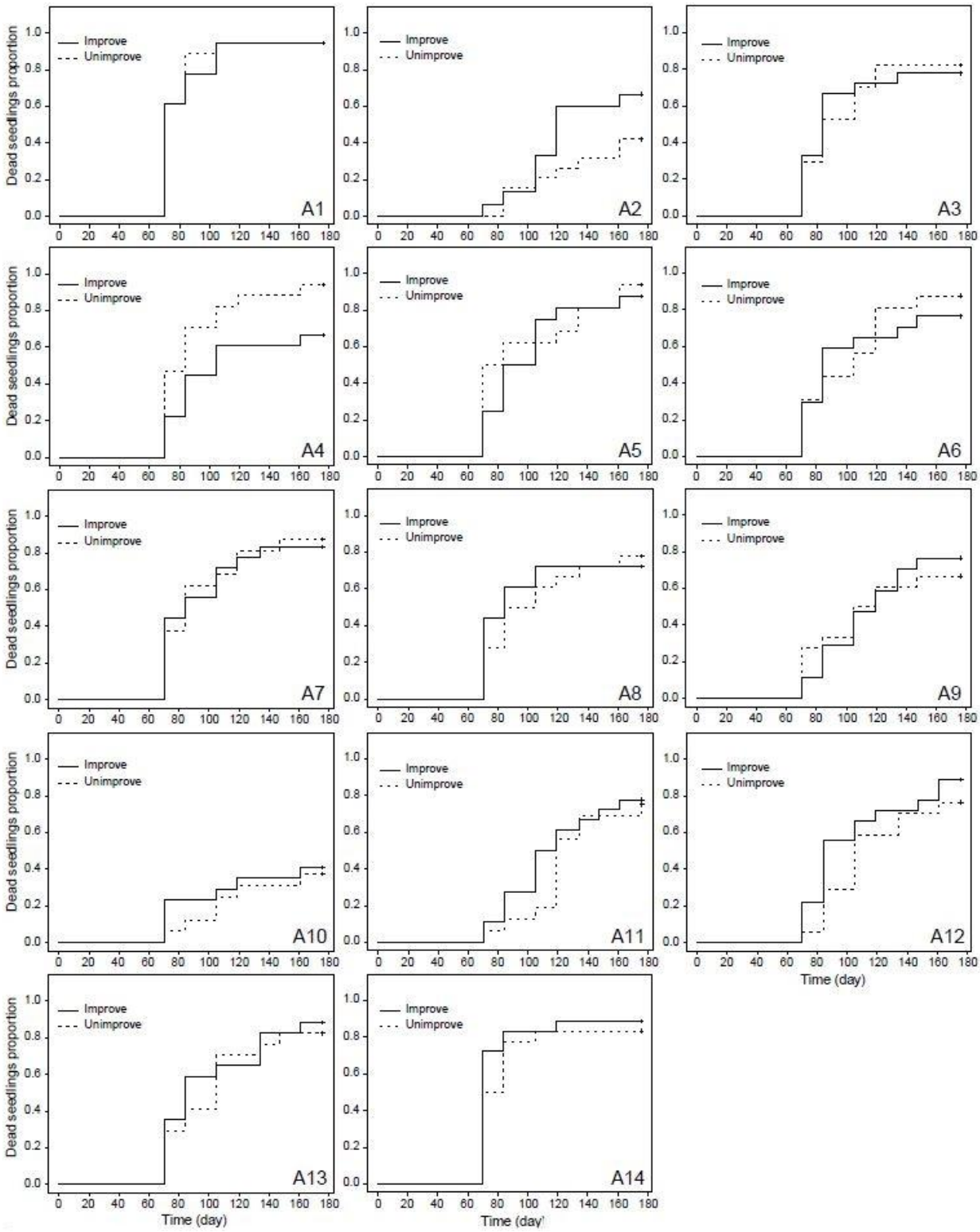

Figure S1: Plot of mortality probabilities (1 - survival probabilities), using the Kaplan-Meier estimate, of *P. pinaster* inoculated with each of the 14 different *A. ostoyae* isolates according to maritime pine provenances (each line type corresponds to a provenance). X axis: days from the beginning of inoculation; Y axis: proportion of dead seedlings.

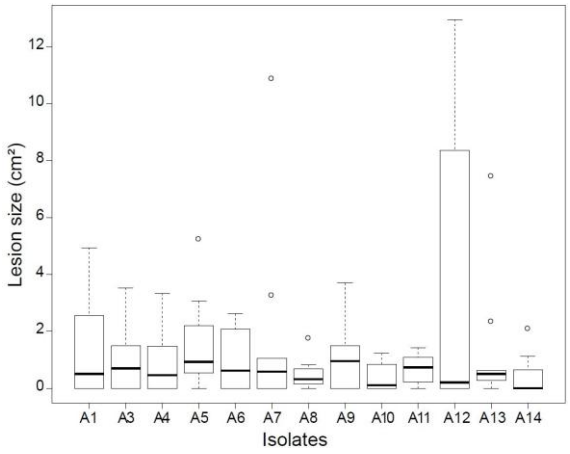

Figure S2: Box plot of the necrotrophic ability per *A. ostoyae* isolates, expressed as the average size of the lesion (cm²).

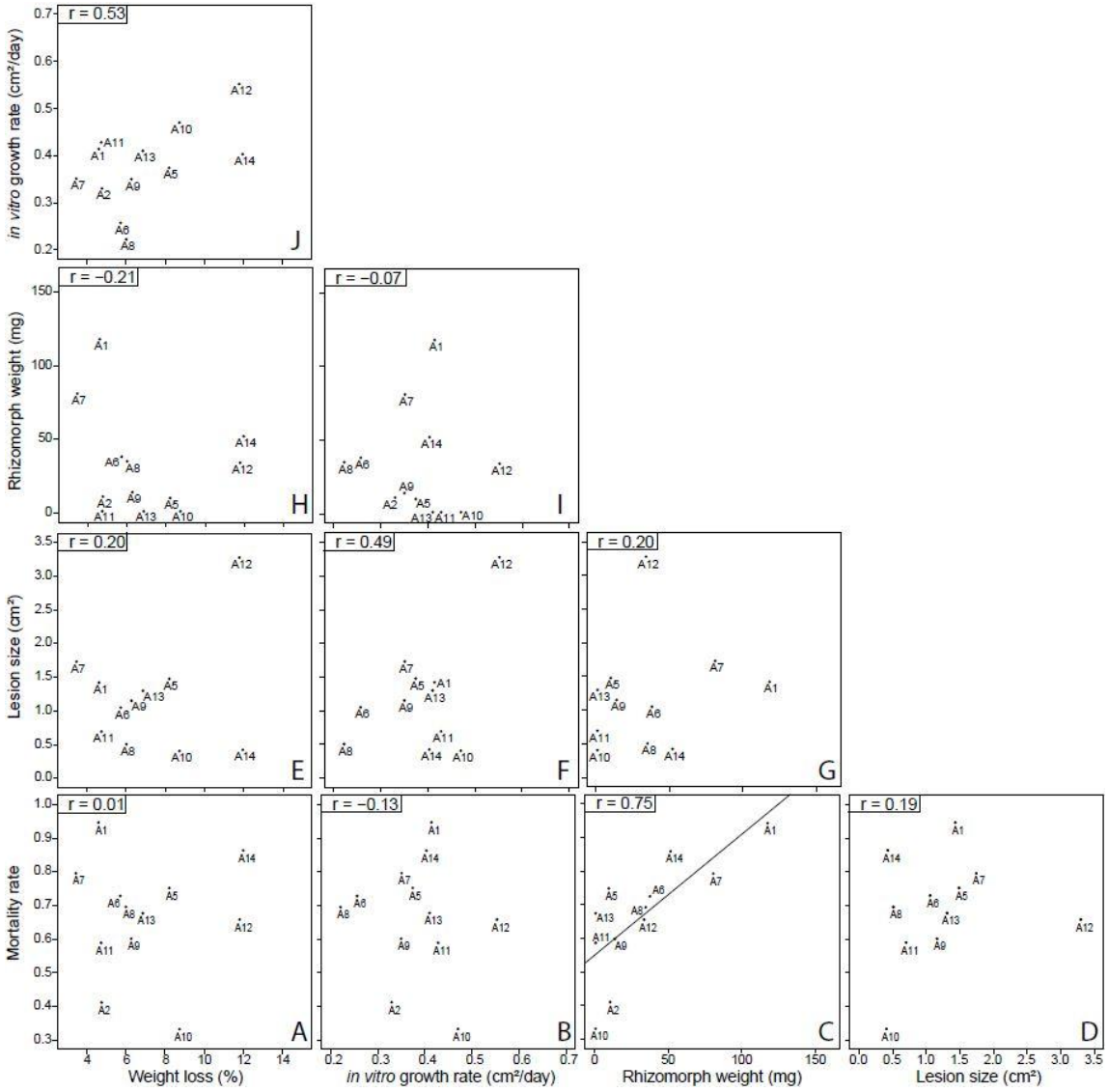

Figure S3: Relationships between the virulence, the rhizomorph production, the *in vitro* growth rate, the necrotrophic and the wood-degrading ability of each 12 *A. ostoyae* isolates sharing the same year of cultivation (2012). The black line represents significant linear regression. Each Pearson correlation coefficient is indicated in the corner of each plot.

ANNEXE CHAPITRE III

No significant variation in virulence between old and new *Armillaria ostoyae* populations in the pine maritime forest of south-western France

Introduction

A. ostoyae is at the origin of significant dieback of the maritime pine (*Pinus pinaster*). For the 30 last years, the number of disease reports has constantly increased in the Landes de Gascogne in the south west of France (Aumonier 2007; Lévy and Lung-Escarmant 1998). The first cases of the disease were reported just after the large plantations in local maritime pine in the second half of the 19th century to develop the local economy and sanitize the marshy soils of the region (Thiveaud 1992; Dupuy 1994; Vallauri et al. 2012; Guyot 1928). Today, one million hectares of the maritime pine monoculture, nearly spatially continuous, allows the increase of pathogen dispersion by favoring connection among forest stands relative to the small and fragmented forests pre-existing before the large plantations. Furthermore, constant host availability in this intensively managed forest with high density of individuals and even-age populations should be favorable factors increasing the disease prevalence as shown in other planted forests (Gerlach et al. 1997; Korhoren 1998; Morrison et al. 1988; Pautasso et al. 2005). A previous epidemiological study has showed the relationships between the current spatial distribution of the disease and the location of the pre-existing forest fragments before the large plantations (Labbé et al. 2015). This result suggests that these ancient forest fragments could be the source of inoculum for the colonization of the pathogen into the new planted areas won on the marshes in the 19th century. Because, pathogens become more virulent as they spread (Phillips and Puschendorf 2013), we hypothesize that *A. ostoyae* isolate became more virulent as they were spreading from the pre-existing forest areas to new planted forest areas. To test this hypothesis we compared the virulence of isolates collected within original forests and in newly planted forests areas.

Materials and methods

3. Host plants

As described in the Chapter III, the plant root inoculations were conducted on 2-year-old maritime pine seedlings. A half of the plants came from an unimproved coastal provenance from the Landes de Gascogne forest (MIMIZ PPA303). The other half of the maritime pine plants correspond to one improved variety of maritime pine (UC106) collected in the seed orchard of the INRA (French National Institute for Agricultural Research) breeding program. At 1-year-old, each bare-root seedling was planted in a 5.5 l plastic cylindrical pot (20 **x** 25.7 cm) containing wood fibre (50%), blond peat (30%), mineral soil (20%) and fertilized with 6 g l^{-1} of slow-releasing fertilizer (Osmocote Exact Lo.Start).

2. *Armillaria ostoyae* isolates

Thirty *A. ostoyae* isolates, sampled on dead maritime pines, according to method described in the chapter III, were tested for their virulence (Figure 2). Out of these samples, fifteen were collected from pre-existing forest areas before plantations, and fifteen from expansion afforested areas (i.e. planted forest areas since the second half of the 19th century). All isolates were sampled between 2012 and 2014. Inoculums were prepared as described by (Guillaumin and Lung 1985)

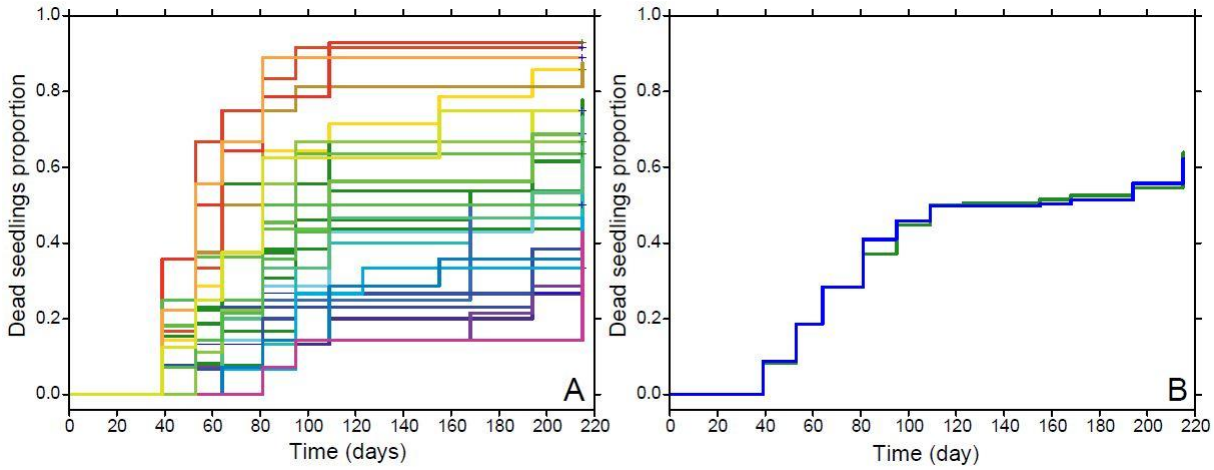

Figure 1: Plot of mortality probabilities (1 - survival probabilities), using the Kaplan-Meier estimate, of *P. pinaster* inoculated with 30 different *A. ostoyae* isolates from pre-existing forest areas (in green) and from new planted forest areas (in blue); A: according to isolates (each different color line corresponds to a tested isolates); B: according to forest area type (each line type corresponds to a forest type: pre-existing forest areas in green and new planted

forest areas in blue). X axis: days from the beginning of inoculation; Y axis: proportion of dead seedlings.

8. Plant roots inoculation

This inoculation was carried out following the protocol described in chapter III, except that each isolate was inoculated in 14 pines plants (i.e. half/half for the improved/unimproved varieties ratio). Overall, 420 plants were inoculated on May 2014.

Results

At the end of the experiment, 68 inoculum sticks (17%) of the survival trees did not contained viable mycelial fans and were discarded. Moreover, among the 420 pine plants inoculated with *A. ostoyae* and 30 control plants, 56 (13%) and 4 (13%) plants died during the experiment with no observation of lesion and mycelium, suggesting a death independent of pathogen infection. These mortalities, not induced by *A. ostoyae*, were also excluded from the analyses. First *A. ostoyae* induced mortalities were observed 40 days after inoculation (Figure 1). The virulence of the 30 *A. ostoyae* isolates from Landes de Gascogne forest was highly variable (log-rank test, *P*-value < 0.001; Figure 1A). The rate of survival of host ranged between 88% and 13% according to the inoculated isolate. However, the comparison of the mean survival of the plants according to the groups of isolates did not allow differentiating isolates from pre-existing forest areas to isolates from newly planted forest areas (log-rank test, *P*-value = 0.90) (Figure 1B).

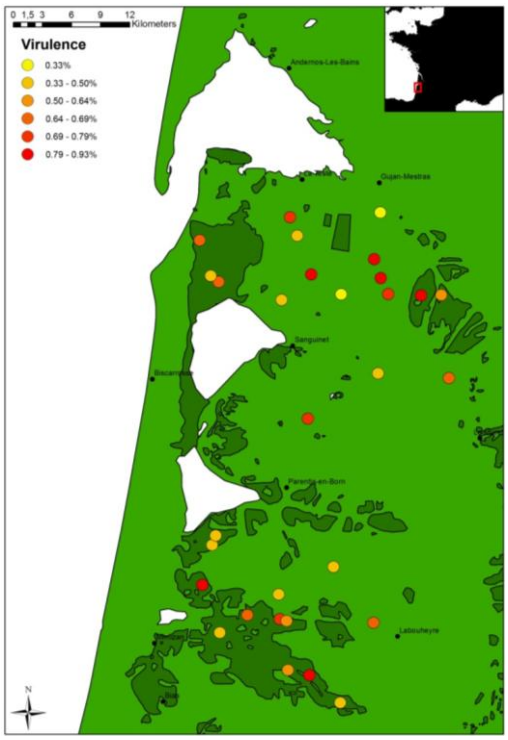

Figure 2: Virulence distribution map of 30 *A. ostoyae* isolates. The original forest areas (Cassini; Vallauri et al. 2012) are indicated in dark green areas.

The virulence of the tested isolates did also not show any geographical structure, with isolates of high and low virulence evenly distributed on the study area (Figure 2). Finally, the host provenance did

not influence the average survival rates caused by *A. ostoyae* (log-rank test, *P*-value = 0.82) (Figure 3).

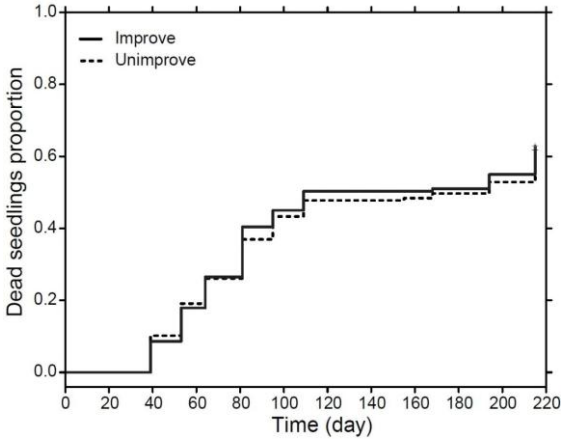

Figure 3: Plot of mortality probabilities (1 - survival probabilities), using the Kaplan-Meier estimate, of *P. pinaster* inoculated with 14 different *A. ostoyae* isolates according to maritime pine provenances (each line type corresponds to a provenance). X axis: days from the beginning of inoculation; Y axis: proportion of dead seedlings.

Discussion

A significant variation of virulence among the 30 isolates was identified in this study, which is consistent with the 14 isolates previously described in the chapter III. However, the virulence would not be dependent on the geographical localization of the *A. ostoyae* isolates. Since there was no significant difference in average virulence between the two groups (i.e. the original forests corresponding to the potential sources of the pathogens and the new planted areas assumed to be more recently colonized by *A. ostoyae*). However, the small spatial scale for which the virulence has been tested might be responsible for these results. Indeed, the new forests of the study area were geographically close to the first reports of the disease, and it is probable that they no longer correspond to recently colonized areas where you could expect a greater virulence of the pathogen.

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CHAPITRE IV

Genetic signatures of variation in population size in a fungal tree pathogen reflect the history of expansion-regression of its host population: the example of *Armillaria ostoyae* in maritime pine forest of south-western France

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Keyword

Approximate Bayesian Computations; expansion; fungal pathogen; generation times; planted forests; root-rot disease

In preparation

Abstract

The root rot fungus *Armillaria ostoyae* (*Basidiomycete*) is one of the major pathogens of conifers in the northern hemisphere. In the forest of the Landes de Gascogne (south-western France), *A. ostoyae* is responsible for significant mortality of maritime pine (*Pinus pinaster*). Phylogenetic and pollen analysis suggest that the pathogen and its host coexisted in this area since several million years. Historical demographic changes of the pine maritime populations, such as the strong reduction of the forest cover during the maximum cooling of the last glaciation (20,000 to 18,000 years before present (BP)), and the massive plantations of maritime pine during the second half of the 19th century, have likely influenced the demographic history of the pathogen. In this study, we used a population genetics approaches to investigate the demographic history of *A. ostoyae* and test which demographic scenarios best explained the genetic polymorphism in this species. Approximate Bayesian Computation (ABC) approach suggested a first population regression, and less strongly, a recent expansion of the fungal effective population size in agreement with the dynamics of the forest of Landes de Gascogne. These results allow us to estimate for the first time for a root pathogen a generation time between 10 and 20 years in this ecosystem.

Introduction

Fungal plant diseases are a major threat worldwide to natural habitat and to economically important crops (see for example Anderson *et al.* 2004; Fisher *et al.* 2012). When exotic (i.e. non-native) fungal pathogens are introduced into new areas they may cause devastating epidemics, and have been intensively studied during the last years (e.g. Desprez-Loustau *et al.* 2007; Gladieux *et al.* 2015). Relative to these introductions, disease emergences due to the demographic expansion of an endemic pathogen have been more rarely investigated. Several studies have revealed that these expansions could be associated with climatic changes favoring opportunistic pathogens (e.g. Rosenzweig *et al.* 2001; Desprez-Loustau *et al.* 2006). However, changes in land use and the intensification of the managements practices could probably more contribute to disease outbreaks (Anderson *et al.* 2004). Many studies on fungal pathogens on crops have described these outbreaks and contributing factors. For example, in response to the growing demand for food resources (Godfray *et al.* 2010), the intensification of agriculture provide a favorable environment for the emergence of pathogens by using higher plant densities, irrigation, fertilization and pesticides (Fowler & Mooney 1990;

Stukenbrock & McDonald 2008). Large scale monoculture favors, first, a rapid spread of the pathogen (Stukenbrock & McDonald 2008), and second, limits biocontrol by natural enemies (Wilby & Thomas 2002; Bianchi *et al.* 2006). Moreover, the increase in use of crop varieties with high yield, generally leads to a strong reduction in the genetic diversity of the planted species, allowing a rapid adaptation and the spread of a limited number of aggressive strains (Edwards 1996; Zhu *et al.* 2000). Due to constant and important development of planted forests often monospecific, for the last 50 years (FAO 2007, 2013), this major environmental change for forest ecosystems would therefore contribute to increase in frequency of tree fungal diseases emergence in the next few years (Delatour *et al.* 1985; Pautasso *et al.* 2005; Ennos 2015; Wingfield *et al.* 2015). Eucalyptus plantations are one of the most impressive examples of this recent phenomenon. Theses plantations, whose surface has almost recently been multiplied by 20 since the mid-20th century, experienced an increase of phytosanitary problems, which have been accelerated over the last 20 years (Wingfield *et al.* 2015).

Numerous studies have retraced the common demographic history of cultivated plants and its associated pathogens (Gómez-Alpizar *et al.* 2007; Zaffarano *et al.* 2008; Gladieux *et al.* 2008). These studies generally highlighted the role of human activities in long distance dispersal of pathogens among continents, sometimes long time after the introduction of their host species in a new geographical area (e.g. Barrès *et al.* 2008; Robert *et al.* 2012; Fontaine *et al.* 2013). This host-tracking by the pathogens may be retraced using population genetic approaches, and it can show that parasites are sometimes excellent proxies to retrace the demographic history of their host (Sugimoto *et al.* 1997; Falush *et al.* 2003; Holmes 2004; Wirth *et al.* 2005). However, studies retracing local expansion of plant pathogens from a native source, for example after a recent development of plantations, have been more rarely investigated. Some recent studies of forest pathogen have described these local expansions (e.g. Dothistroma needle blight (*Dothistroma septosporum*) on *Pinus contorta*; Woods *et al.* 2005), but sources of the expansion, effect of large monospecific plantations in the native area, and timing of the expansion have been rarely identified. Actually, such studies of current expansion associating host and its native pathogen may be difficult to retrace using population genetic studies because of several limitations. First, only few (even one single) genotypes may be at the origin of the outbreaks independently of the genetic diversity of the host (e.g. Raboin *et al.* 2007). In this context, it may be

difficult to estimate variations in population size associated with this expansion, and genetic studies of pathogen populations are of little interest to reflect the host demography. Second, very recent population expansions are usually much more difficult to detect from genetic data as not enough new mutations can accumulate within a few generations after population growth (Girod *et al.* 2011). Furthermore, forest distribution have dramatically varied during geologic era, particularly because of environmental changes during the last glaciations (see for example in Europe: Taberlet *et al.* 1998; Hewitt 2000; Petit *et al.* 2003). Genetic signals of rapid current pathogen expansions may therefore be masked by these strong variations of population size occurring during past demographic changes in their hosts, if they have an ancient common history.

The maritime pine forest of the Landes de Gascogne, located in south-western France (Figure 1), is a textbook example of a forest having experienced strong cover changes over time, both during ancient environmental change and during recent man-made activity (Frenzel *et al.* 1992; Jolivet *et al.* 2007; Temple 2011). During the height of the last glaciation (20,000 to 18,000 years BP), the distribution of conifer forest were extremely reduced in that part of Europe (Frenzel *et al.* 1992) and was mainly composed of periglacial tundra and boreal forests (i.e. mixed and coniferous forests) only in the southern part of Europe. During the post-glacial warming (10,000 years BP), gradual increase in temperatures contributed to the progressive recolonization of the forest in this area from the potential Iberic refugia (Taberlet *et al.* 1998). Pollen analysis witnessed the existence occurrence of maritime pine in this area since the Boreal Period (8,800 to 7,500 years BP; Paquereau 1964). However, the increase in human activities during the Subatlantic Period (2,700 years BP) led to another reduction in the forest distribution in the south-west region of Europe mostly for the purposes of agriculture and the manufacture of dwellings. The forest cover in the Landes de Gascogne remained weak until the large plantations of the second half of the 19th century (Jolivet *et al.* 2007). In order to develop the local economy and to sanitize the moors and marshes, which mainly composed this area, French government enacted in 1857 a law which required to drain and forest the communal pastures with a local species, the maritime pine (*Pinus pinaster*) (Thiveaud 1992; Dupuy 1994; Temple 2011). Therefore, the initial forest only composed on few small forest fragments (generally smaller than 300 ha), and mainly located along the Atlantic coast (Vallauri *et al.* 2012), increased to less than 250,000 ha to nearly 750,000 ha in only 50 years. It represents now a million

of hectares of pine monoculture, becoming one of the largest contiguous monospecific forests in Europe.

Shortly after this significant change in the landscape (in 1920), the first cases of maritime pine mortality caused by the fungal root pathogen, *Armillaria ostoyae* were detected near the Atlantic coast (Guyot 1928). *Armillaria ostoyae* (Romagn.) Herink, is one of the most aggressive *Armillaria* species for conifers of the northern hemisphere causing serious damages to conifers forest, especially in the Landes de Gascogne on maritime pine (Guillaumin *et al.* 1993). For the nearly last thirty years, regular monitoring of the disease occurrence by the French Health Department of Forests (DSF) and the French National Institute for Agricultural Research (INRA), showed increasing incidence of the pathogen in the Landes de Gascogne (Lévy & Lung-Escarmant 1998; Aumonier 2007). Nowadays, the disease is mainly distributed along the Atlantic coast and within or near forest areas pre-existing before the large pine plantations of the second half of the 19th century (Labbé *et al.* 2015). This current spatial distribution of the disease supported the hypothesis that *A. ostoyae* disease is emerging from these pre-existing forests which would be an inoculum reservoir for the colonization of new planted forests. Furthermore, the gradient of allelic richness of *A. ostoyae* populations, decreasing from west coast to the east of the massif (Prospero *et al.* 2008), is in agreement with a fungal expansion from these initial forest sources mainly located in the west (Vallauri *et al.* 2012).

The previous study (Prospero *et al.* 2008) showed a decreasing genetic diversity from west to east suggesting a Eastward expansion of the pathogen by successive steps of founder events. However the limited sampling previously analyzed (only 31 disease foci) limited our ability to understand the origin(s) and importance of *Armillaria* expansion from one main or multiple local sources. In this study, we used a dense geographic sampling of the south west region of France to re-assess the genetic diversity of *A. ostoyae* and analyzed how it is structured in space. Our first objective is to assess the degree of genetic differentiation among *A. ostoyae* foci present in the forests pre-existing before plantations and in the pine plantations established on the drained marsh, these latter being associated with the expanding *A. ostoyae* population. From this genetic structure, we determined whether *A. ostoyae* expansion in south west France resulted from only one or multiple genetically divergent sources. The second objective was to infer the timing and the intensity of the demographic events using an approximate Bayesian computation (ABC) approach (Beaumont *et al.* 2002; Csilléry *et al.* 2010).

According to the importance of the presence of maritime pine for its parasitic stage in the Landes de Gascogne (Guillaumin *et al.* 1993; Lung-Escarmant & Guyon 2004), the pathogen demographic history should be strongly associated with both the past demographic regressions and the recent expansion of its host. Assuming this hypothetical scenario is correct, past and recent demographic changes in effective population size should detectable by analyzing the genetic structure of *A. ostoyae* populations.

Materials and methods

1. Biological characteristics of the pathogen species

A. ostoyae is a basidiomycota from the agaricale family. Phylogenetic analyses estimated that the most recent common ancestor of *A. ostoyae* emerged around 7 million years BP (Coetzee *et al.* 2011). At our knowledge, there is no fossil data for *A. ostoyae* in Europe. However, this ancient emergence and the presence of maritime pine in the south-western France for several million years BP, may suggest that the two species could have co-existed long times before the last glaciations. The fungus can infect pine roots by using differentiated vegetative structures called rhizomorphs, which are formed in infected roots and can extend through the soil to reach new hosts (Day 1927; Thomas 1934). Infection may also occur through root contacts (Zeller 1926; Childs & Zeller 1929). This short distance transmission result in disease foci composed on one or few clonal genotypes which can sometimes reached few hectares in the Landes de Gascogne (Prospero *et al.* 2008) and hundreds of hectares in North America (Smith *et al.* 1992; Ferguson *et al.* 2003). *A. ostoyae* behaves as a necrotrophic parasite on pines, which colonises and kills the root tissues, and as a saprobe which can develop on dead tissues and can persist for several years in soil residuals after plant clearing, as described for *A. mellea* (Rishbeth 1972). Colonization of new stand from initial disease foci is still poorly understood. In autumn, *A. ostoyae* fruiting bodies produce basidiospores, and dispersed by wind infecting fresh wood substrate (e.g. stumps and partly buried stem segments), usually a few hundred meters away (Legrand *et al.* 1996; Hood *et al.* 2008). According to previous studies (Rishbeth 1988; Legrand *et al.* 1996; Prospero *et al.* 2008; Dutech *et al.* submitted), the mechanism of spore-dispersal seems to contribute to the spread of *A. ostoyae.* However, both the difficulty to observe the germination of basidiospores in the field (Rishbeth 1988), and the low number of new recorded disease foci for 30 years in the Landes de Gascogne (Aumonier 2007; Labbé *et al.* 2015) suggest that the establishment of these new disease foci is a rare event in

forest. Assuming a weak colonization ability of the pathogen by the spread of basidiospores, it is not clear how large the *A. ostoyae* populations have increased during the last 150 years after the development of pine plantations.

2. Study area and sampling

The study area is located in the Landes de Gascogne forest in the south west of France and covered about a quarter of the current forest (240,000 ha). It encompassed both ancient (anterior to 1857; Vallauri *et al.* 2012) and new afforested areas (after 1857; Figure 1). Previous studies have shown that many disease foci with the highest genetic diversity were observed in this area relative to other parts of the forest (Prospero *et al.* 2008; Dutech et al. submitted). This high occurrence of *A. ostoyae* allows us to benefit of a sample with several different genotypes needed for population genetic studies and inferences of historical demography of the pathogen. Two hundred twenty-one samples of subcortical mycelium (86%) or fruiting bodies (14%) of *A. ostoyae* were collected between 2012 and 2014 from dead and dying maritime pines (Figure 1). These trees samples were spaced at a minimum distance of 100 m to reduce the possibility of sampling *A. ostoyae* clonal genotypes associated with the same disease focus that commonly observed at this spatial scale (Prospero *et al.* 2008). For each field sample, some mycelium (around 2 mg) was removed, cleaned for dusts and piece of wood in the laboratory, then lyophilized in a microtube during 12 hours at -45°C and 0.3 mbar, and stored at -80°C until the DNA extraction.

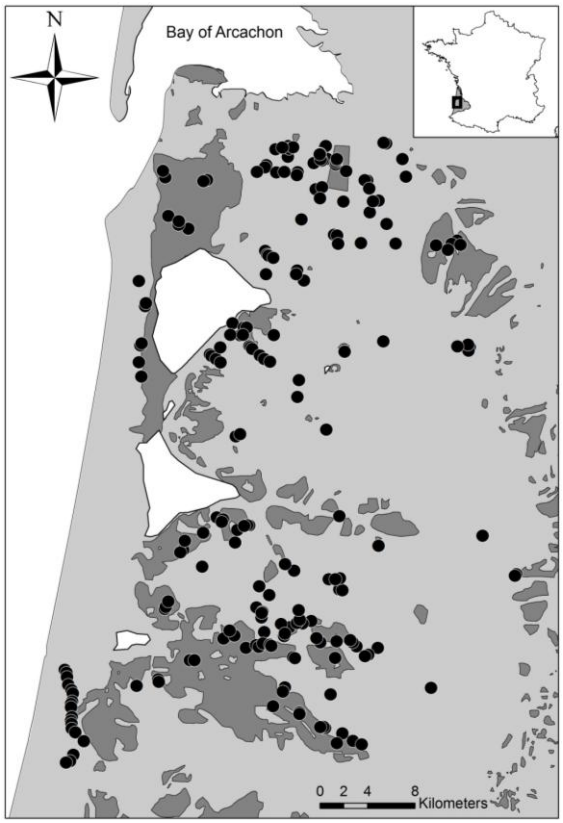

Figure 1: Geographical location of the *A. ostoyae* samples. The black dots indicated subcortical mycelium or fruiting bodies samples of *A. ostoyae* collected on dead and dying maritime pines. The pre-existing forest areas (Cassini) are indicated in dark grey areas. The Landes de Gascogne forest is indicated in light grey on the map of France.

3. DNA extraction, microsatellites and SNPs genotyping

Total fungal DNA was extracted from lyophilized mycelium with cetyltrimethyl ammonium bromide (CTAB) extraction

buffer, according to the protocol of Prospero *et al.* (2008). The extraction products were purified with the innuPREP PCR pure kit (Analytik Jena, Biometra, Germany) and stored at -20°C. The concentration of DNA was determined on a NanoDrop spectrometer (NanoDrop Technology, San Diego, CA, USA) and was adjusted to 10 ng/µL using a STARTlet 8-channel robot (Hamilton, USA).

A. ostoyae individuals (i.e. mycelium samples) were genotyped using 14 polymorphic microsatellite loci: AoSSR21a, AoSSR74a, AoSSR75a (Langrell *et al.* 2001), CAG25a, CAG77a (Worrall *et al.* 2004; Prospero *et al.* 2008), Arm05, Arm09, Arm15, Arm16 (Prospero *et al.* 2010), and finally, AoB8A4Z, AoB8PN1, AoB9MK4, AoCE9NK, AoCFZOL (Malausa *et al.* 2011). We designed two multiplexes which co-amplified seven microsatellite loci each (Table S1). For the multiplex polymerase chain reaction (PCR), we used the Qiagen Multiplex PCR kit. For both multiplexes, PCR mix was composed of 3 µL of sterile water, 4 µL of Qiagen Multiplex Buffer (2X), 2 µL of primer premix (primer pairs concentrations indicated in the table S1) and 3 µL of DNA (10 ng / µL). PCR cycling was carried out using a Labcycler 48 thermocycler (SensoQuest Biomedical Electronics). Thermal cycler conditions were the same for the two different multiplexes: initial denaturation at 95°C during 15 min; followed by a total of 34 cycles of denaturation at 94°C for 30 s, primer annealing at 55°C for 1 min and extension at 72°C for 45 s; and a final extension at 60°C for 30 min. After testing the successful amplifications of the PCR products on 1% agarose gels stained with GelRed (Biotium, USA), genotyping was performed on a capillary sequencer (ABI 3730; Applied Biosystems, USA). Genotyping results were then analysed and manually inspected with the STRand v.2.4.59 software (Toonen & Hughes 2001). Individuals with unclear genotypes were genotyped twice.

In addition to the microsatellite loci, the samples were also genotyped at 27 SNPs loci (Dutech *et al.* submitted). SNPs genotyping was performed using the MassARRAY Analyser 4 system (Agena Bioscience, San Diego, USA) according to the iPLEX protocol from Sequenom (Gabriel *et al.* 2009). The resulting data were analysed and inspected manually using the MassARRAY Typer Analyzer v.4.0 software (Agena Bioscience, San Diego, USA).

4. Data analyses

a. Genetic diversity

Clonal genotypes present in the sample were identified using GENCLONE software v.2.0 (Arnaud-Haond & Belkhir 2007). Genetic analyses were therefore performed with only one representative individual for each genotype to remove the

effect of clonal structure on the analysis. Genotypes with more than 5 missing alleles (30% NA) were discarded. Linkage disequilibrium among loci was tested using a permutation test (1,000 permutations) implemented in GENEPOP v.4.2 (Raymond & Rousset 1995; Rousset 2008). For each locus, fixation index (F_{IS}, Wright 1969), genetic diversity (H_e, Nei 1973) and allelic richness (A_r) were estimated using GENEPOP v.4.2. Departure of the F_{IS} values from zero was also tested with GENEPOP using an exact test (499 permutations). The nominal *P*-value of 0.05 was adjusted for multiple comparisons using a false discovery rate (FDR) correction (Benjamini & Hochberg 1995) and performed on R v.2.15.1 statistical software (R Core Team 2012). We used micro-checker v.2.2.3 software (Van Oosterhout *et al.* 2004) to check the occurrence of null alleles and possible genotyping errors in the data. A spatial genetic signal of the pathogen expansion from the pre-existing forests were looked for, using the geographic distribution of the genetic diversity (H_e and A_r). It was estimated by computing the spatial interpolation of the local H_e and A_r values (i.e. estimated for 16 subparts (14,000 ha) of the study area), using the thin-plate spline method implemented in the R package fields (Furrer *et al.* 2011). We also estimated the inter-population fixation index (F_{ST}), using Weir & Cockerham (1984) method, implemented in GENEPOP v.4.2. Genetic differentiation among groups of isolates from different geographical areas (i.e. north, east, south and west, and from the pre-existing forest areas and the new planted areas was tested with an exact test (1,000 permutations) using GENEPOP program. Due to the slight inaccuracies of the Cassini map georeferencing (Vallauri *et al.* 2012), only some groups of individuals not closer than 500 m from the borders of the pre-existing forest areas, were retained for the composition of these groups.

b. Population genetic structure

We first investigated the genetic structure without a priori knowledge using two individual-based methods. We first applied a Bayesian model-based individual clustering method implemented in STRUCTURE v2.3.4 (Pritchard *et al.* 2000) to investigate the population structure of *A. ostoyae*. This analysis assigns individuals to genetic clusters (*K*) while minimizing departure from Hardy Weinberg Equilibrium (HWE) and linkage disequilibrium among loci within each cluster. The analysis was conducted first using an admixture model, assuming correlated allele frequencies among populations and uniform priors for the population of origin of each individual. We also used a more recent admixture model which uses the sampling location as *a prior* information in the Bayesian inference designed (*Locprior* model). This model has

better performance to detect existing genetic structure when the level of divergence is weak or when limited amount of information is available, yet without introducing biases towards detecting structure when it is not present (Hubisz *et al.* 2009). For each analysis, we performed a series of independent runs with different values for the number of *K*, testing all values from 1 to 10. Each run was conducted with 500,000 Markov chain Monte Carlo (MCMC) iterations after a burn-in period of 50,000 MCMC iterations. We conducted 10 independent replicates for each value of *K* to ensure convergence of the MCMC. We identified the number of clusters (*K)* that best explains the data first by computing the posterior probability of the data (Ln(Prob(*X*|*K*) also know as Ln(D), Pritchard *et al.* 2000) for each number of assumed *K* following the STRUCTURE user guide and; second by computing the rate of change of these values as *K* increased (Evanno *et al.* 2005). To assign groups of runs to a common clustering pattern, we computed the symmetric similarity coefficient between pairs of runs (100 random input sequences, G' statistic) with CLUMPP v.1.1 (Jakobsson & Rosenberg 2007) using the Greedy algorithm. Barplots were then generated from CLUMPP outputs for each *K* value with DISTRUCT v.1.1 (Rosenberg 2004).

The second type of methods we applied are multivariate analyses of genetic data that provide a complementary view to model-based Bayesian clustering approach (Jombart *et al.* 2009; McVean 2009) independently of any model assumptions (François & Durand 2010). We further analysed the genetic structure using a Principal Component analyses (PCA) (Patterson *et al.* 2006; Novembre & Stephens 2008; McVean 2009) and a modified version of this analysis, known as spatial PCA or sPCA (Jombart *et al.* 2008), accounting for spatial autocorrelation, and aiming at displaying genetic variance with a spatial structure. The sPCA scores reveal two types of pattern, defined as global and local structures (Thioulouse *et al.* 1995). Global structures correspond to positive spatial autocorrelation (i.e. neighbouring genotypes are more similar than expected), whereas local structures correspond to negative spatial autocorrelation (i.e. neighbouring genotypes are more dissimilar than expected). We investigated the global and local structures, as described by Jombart *et al.* (2008) by testing the significance of the spatial principal components of the sPCA using 10,000 permutations. Each significant structure detected was displayed by plotting the samples according to their geographic coordinates, colouring and sizing them according to their respective scores along the sPCA component. Following Jombart *et al.* (2008), the data were centred and scaled before analysis and the missing

alleles were replaced by the mean allele frequencies estimated from the whole data.

Since the genetic structure of *A. ostoyae* at this spatial scale could be mostly due to the equilibrium between limited gene dispersal and genetic drift, we also tested the occurrence of an isolation by distance (IBD) pattern in which individuals geographically closer are more likely to be genetically more similar to each other than more distant individuals (Wright 1943; Rousset 1997). To test that hypothesis, we analyze the spatial correlation between genetic similarity and geographical distance among *A. ostoyae* isolates by performing the regression analyses of pairwise kinship coefficients (F_{ij}) estimated between pairs of isolates (Loiselle *et al.* 1995) on spatial distances separating them. We constructed the spatial correlogram by plotting the average F_{ij} as a function of the spatial distance class, with the software SPAGeDI v.1.5a (Hardy & Vekemans 2002). Classes of spatial distances were defined for each kilometer until 40 km, and each two kilometers beyond 40 km with the last distance class being separated by 58 to 60 km. In addition, F_{ij} values were regressed on the natural logarithm of the spatial distance between individuals in order to test for isolation by distance model, and assuming a gene dispersal in a two dimensions space (Rousset 1997). Permutation tests (10^4 permutations of individual spatial locations) were performed to test for the significant departure from zero of the regression slope between the kinship coefficient and the geographic distance at each distance class (Rousset 1997). Following Vekemans & Hardy (2004), we also quantified the intensity of the spatial genetic structure with the parameter $Sp = -b/(1 - F_1)$, where b is the slope of the regression of F_{ij} on log(spatial distance separating pairs of isolates), and F_1 is the mean F_{ij} between pairs of individuals in the first distance class (i.e. 0 to 1 km).

5. Demographic history

Finally, we investigated which demographic history best describe the genetic diversity of *A. ostoyae* in the Landes de Gascogne forest using the ABC approach implemented in the computer program DIYABC v2.0.4 (Cornuet *et al.* 2008, 2010, 2014). This coalescent-based method simulates millions of datasets comparable to our observed genetic data under alternative demographic model and compares them to identify which model best explain the observed data. Once a demographic model has been selected, DIYABC can estimates the parameter values of interest.

We tested nine possible scenarios of demographic changes (Figure 2). The first scenario (SC1) consisted of a null hypothesis and assumed that the effective size (N_1) of the *A. ostoyae* population remained constant over time, irrespective

of any changes in the forest surface. In contrast, the other eight scenarios tested consist of changes in the *A. ostoyae* population effective size that can be related to different combination of surface expansion or contraction of the forest, and consequently of the host population size. The second (SC2) and fourth (SC4) scenarios assumed that the ancestral effective size population (N_a) decrease t_1 or t_2 generation ago, to reach its current effective size (N_1). Under these two scenarios, t_1 and t_2 correspond respectively to the time of the ancient significant decrease of the forest surface during the last glaciation and to time of the recent increase of the forest surface during the massive plantation of the second half of the 19th century. In the later scenario (SC4), the decrease of *A. ostoyae* populations might be associated with a colonization of new pine plantations by a limited part of the fungal ancestral population. The third (SC3) and fifth (SC5) scenarios assumed that the ancestral population effective size (N_b) increased t_1 or t_2 generation ago to reach its current effective size (N_1). The scenario SC6 and SC7 assumed that the effective population size (N_1 and N_a respectively) was reduced t_1 generations ago to a lower size (N_b) and increased t_2 generations ago to reach its current effective size (N_1). These two scenarios are similar, except for the ancestral and present population effective size. In SC6, the present effective population size (N_1) is the same as the ancestral population size, whereas in SC7 the ancestral population size (N_a) was higher than the present (N_1). The scenario SC8 and SC9 assumed the reverse situation: the effective population size (N_1 and N_b respectively) increased t_1 generations ago to a larger size (N_a) and was then reduced t_2 generations ago to reach its current effective size (N_1). As for the scenarios SC6 and SC7, these two scenarios differed only in ancestral and present population effective size.

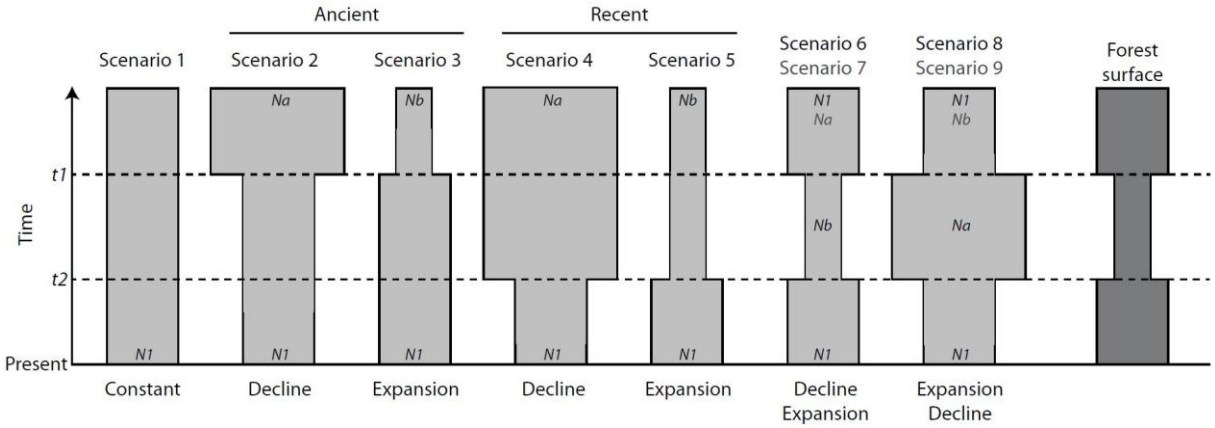

Figure 2: Graphical representations of the nine scenarios of *A. ostoyae* population size evolution in the Landes de Gascogne forest compared by the ABC approach. The time scale is indicated by the arrow on the left. The time was measured backward in generations before the present. Further details of each scenario parametrization are provided in Table S2.

For each of the nine scenarios considered, we simulated 10^6 datasets using the coalescent framework of DIYABC. The parameters that define each scenario were considered as random variables for which priors distributions must be defined, as shown in Table S2. We assumed a generalized stepwise mutation (GSM) model for the coalescent simulations of the microsatellite loci (Estoup *et al.* 2002), with two parameters: the mean mutation rate (μ) and the mean parameter of the geometric distribution used to model the length of mutation events (P) drawn from uniform prior distributions (μ: [10^{-5}–10^{-3}] and P: [0.1–0.3], Table S2). Each locus had a possible range of 40 contiguous allelic states. Individual mutation rates for each locus (μ_{loc}) and the geometric distribution parameters (P_{loc}) were drawn from gamma distributions with respective means μ and P, and shape parameter 2 (Verdu *et al.* 2009). We also considered the possibility of single nucleotide insertion or deletion mutations in the microsatellite sequence. We used default values for other mutation model settings (Table S2). The summary statistics used in this ABC analysis consist in the mean number of allele per locus (A), the mean expected heterozygosity (H_e) and the mean allele size variance (V) over all loci. The posterior probability of each competing scenario was estimated using a polychotomous logistic regression (Cornuet *et al.* 2008, 2010) on the 1% of simulated datasets closest to the real dataset. We evaluated the power of our ABC analysis to discriminate between scenarios, by analysing simulated datasets with the same number of loci and individuals as our real dataset. As described by Cornuet *et al.* (2010), we estimated the Type I error probability as the proportion of instances in which the selected scenario did not exhibit the highest posterior probability among the competing scenarios, for 500 simulated datasets generated under the best-supported scenario. Similarly, we estimated the Type II error probability, by simulating 500 datasets for each of the other eight alternative scenarios and calculating the mean proportion of instances in which the best-supported model was incorrectly selected as the most probable scenario. Finally we conducted a model checking procedure implemented in DIYABC to evaluate the goodness-of-fit between the posterior parameter distribution and the observed data following Gelman *et al.* (1995). For this analysis, we simulated 1,000 pseudo-observed datasets under each studied model-posterior combination, with sets of parameter values drawn with replacement among the 1,000 sets of the posterior sample. This generated a posterior cumulative distribution function for each summary statistic, allowing us to estimate the P-values for the observed values of these summary statistics. We evaluated the

ability of our ABC approach to discriminate between scenarios, by analysing simulated datasets with the same number of loci and individuals as our real dataset. As described by Cornuet *et al.* (2010), we estimated the Type I error probability as the proportion of instances in which the selected scenario did not exhibit the highest posterior probability among the competing scenarios, for 500 simulated datasets generated under the best-supported scenario. Similarly, we estimated the Type II error probability, by simulating 500 datasets for each of the other eight alternative scenarios and calculating the mean proportion of instances in which the best-supported model was incorrectly selected as the most probable scenario.

Results

1. Genetic diversity

Out of the 221 individuals genotyped, only two of them (separated by 143 m) had the same multilocus genotype and were considered as clonal genotype. After keeping only one representative sample of this clone, and removing the genotypes with more than 5 missing alleles (i.e. 6% of the genotypes), the dataset was reduced to 206 individuals, with less than 3.5% missing data. The mean expected heterozygosity (H_e) was 0.53 (SE: ± 0.06) and ranged from 0.02 to 0.82 for the microsatellite loci and was 0.35 (SE: ± 0.03) and ranged from 0.09 to 0.50 for the SNPs loci (Table 1). The mean allelic richness value was 5.86 (SE: ± 0.77) and ranged from 2 to 10.62 for the microsatellite loci. Out of the total 41 loci analysed, nine loci (seven microsatellite and two SNP loci) displayed significant deficit in heterozygote compared to what would be expected under Hardy-Weinberg equilibrium (HWE). The micro-checker analysis revealed that these markers were potentially affected by the occurrence of null alleles. As the occurrence of null alleles may bias genetic estimates, these loci were not retained in subsequent analyses (Table 1). Significant linkage disequilibrium was also detected for three SNP loci pairs: FG716_1 and FG716_8; FG771_1 and FG771_3; FG848_6 and FG848_6). One SNP for each pair was excluded from the subsequent analyses (FG716_8, FG771_1 and FG848_6). Without these loci, the mean estimate of F_{IS} was 0.02 (SE: ± 0.01) and was not significantly different from 0 (*P*-value = 0.09), showing no significant departure to the estimate expected under HWE.

2. Genetic structure

PCA did not suggest any genetic clusters among the multilocus *A. ostoyae* genotypes (Figure S1). This was consistent with no significant differences in allelic frequencies among the four geographical groups, or comparing between genotypes

Locus		*NA* (%)	A_r	H_e	H_o	F_{IS}
Microsatellite	AoB8A4Z	16.0%	10.24	0.804	0.734	0.087
	AoB8PN1 †	3.9%	2.00	0.243	0.000	1.000***
	AoB9MK4	7.8%	3.47	0.457	0.432	0.055
	AoCE9NK †	23.3%	4.93	0.375	0.082	0.781***
	AoCFZOL †	6.3%	2.45	0.016	0.005	0.666***
	AoSSR21a †	43.2%	7.00	0.706	0.368	0.480***
	AoSSR74a †	19.9%	10.62	0.821	0.358	0.565***
	AoSSR75a †	7.8%	8.60	0.640	0.368	0.425***
	Arm05	3.4%	3.97	0.599	0.618	-0.033
	Arm09 †	3.9%	8.42	0.627	0.409	0.348***
	Arm15	2.9%	5.00	0.559	0.490	0.123
	Arm16	8.3%	7.83	0.720	0.714	0.008
	CAG25a	11.2%	4.64	0.564	0.525	0.069
	CAG77a	6.8%	2.85	0.304	0.318	-0.045
SNP	FG487_1	0.0%	2.00	0.361	0.413	-0.144
	FG524_3	1.0%	2.00	0.358	0.368	-0.027
	FG529_1	7.8%	2.00	0.342	0.311	0.093
	FG543_5	0.0%	2.00	0.403	0.403	0.001
	FG652_1	1.9%	2.00	0.497	0.505	-0.016
	FG686_1	1.0%	2.00	0.304	0.304	-0.000
	FG691_1	22.3%	2.00	0.498	0.488	0.021
	FG692_2	3.4%	2.00	0.408	0.407	0.001
	FG698_3	1.0%	2.00	0.491	0.515	-0.048
	FG716_1	1.0%	2.00	0.265	0.294	-0.109
	FG716_8 †	1.0%	2.00	0.262	0.230	0.120
	FG730_3	1.5%	2.00	0.351	0.335	0.047
	FG735_1	1.0%	2.00	0.484	0.451	0.068
	FG747_4	0.5%	2.00	0.207	0.234	-0.130
	FG756_1	0.0%	2.00	0.500	0.485	0.029
	FG762_5	1.0%	2.00	0.295	0.299	-0.015
	FG771_1 †	1.0%	2.00	0.310	0.294	0.051
	FG771_3	1.0%	2.00	0.464	0.412	0.112
	FG788_1 †	1.0%	2.00	0.128	0.088	0.312***
	FG848_1	0.0%	2.00	0.323	0.316	0.022
	FG848_6 †	1.0%	2.00	0.216	0.206	0.045
	FG893_1	1.5%	2.00	0.354	0.340	0.040
	MS334_3	0.0%	2.00	0.401	0.398	0.008
	MS441_2	0.5%	2.00	0.229	0.215	0.064
	MS452_3	0.0%	2.00	0.350	0.325	0.072
	MS467_8 †	17.0%	2.00	0.444	0.181	0.592***
	MS481_1	0.5%	2.00	0.089	0.093	-0.046
All	Multilocus	5.7%	3.32	0.409	0.349	0.146***

Table 1: Genetic diversity and fixation indices at the 14 microsatellite and 27 SNP loci for *A. ostoyae* from the south-western France. *Na* (%): mean percentage of missing allele; A_r: allelic richness; $H_{e:}$ unbiased expected heterozygosity (Nei 1973); $F_{IS:}$ fixation index (*: $P \leq 0.05$; ** ≤ 0.01; *** < 0.001). †: loci excluded from analyses.

for pre-existing forest areas and areas afforested after 1857 (F_{ST} = 0.004, *P*-value = 0.40). Similarly, we did not observe any population subdivision using the Bayesian model-based clustering analysis implemented in STRUCTURE, even with the *locprior* admixture model (Figure S2). The estimated probability data of including only one cluster (*K*=1) was 100% for both the standard and *Locprior* model (Figure S2).

The map of interpolated genetic diversity shows hot spots of diversity close to the largest pre-existing forest areas, as well as a west to east gradient with a lower diversity in the east (Figure 3). The first two principal component of the sPCA showed little spatial genetic subdivision between the multilocus genotypes, with a gradient similar to the geographic distribution of the genetic diversity (Figure S3).

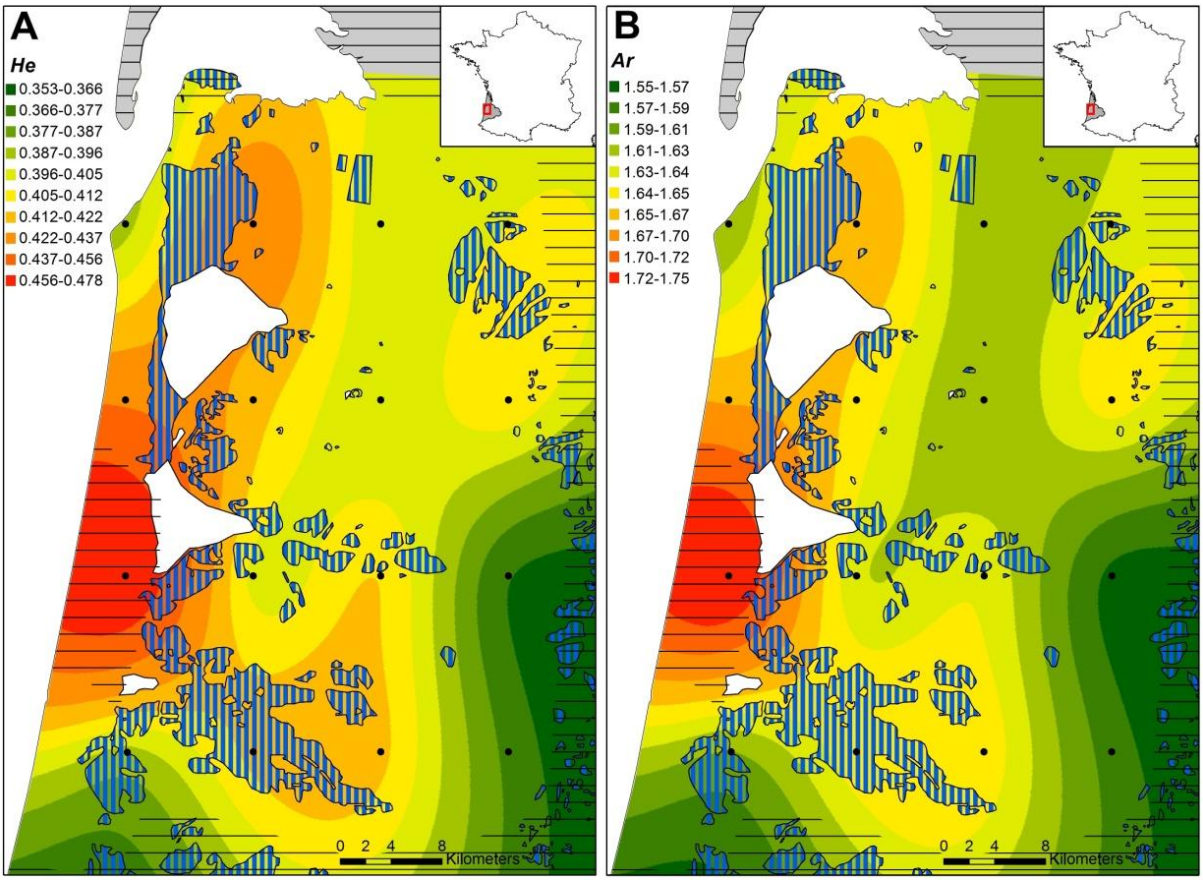

Figure 3: Spatial interpolation of the genetic diversity of *A. ostoyae* in the Landes de Gascogne forest. A: for the genetic diversity (H_e) (Nei 1973); B: for the allelic richness (A_r for a standardized sample size of 3). Black dots indicated the center location of each 16 subparts of the study area, and unsampled areas are indicated by horizontal hatching.

However, statistical tests for both the global and local structures were not significant (*P*-value = 0.59 and *P*-value = 0.30, respectively). Overall, all these results suggest very little or no genetic structure among geographic areas, which mean that all *A. ostoyae* genotypes can be considered as coming from a single panmictic population.

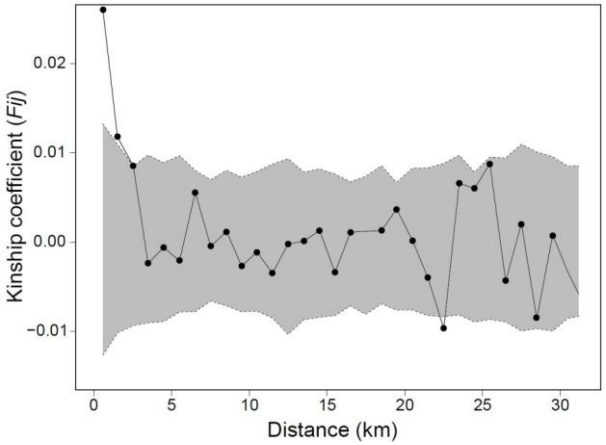

Figure 4: Correlogram of kinship coefficients (*Fij*) among pairs of *A. ostoyae* multilocus genotypes as a function of pairwise spatial distance classes. The grey area indicates values not significantly different from those obtained for a random structure ($P > 0.05$; obtained after 1,000 permutations of spatial location).

Under isolation by the distance (IBD), the estimated kinship coefficient (F_{ij}) should decrease with the spatial distances separating the pairs of *A. ostoyae* individuals (Figure 4). We detected a significant IBD only for the first two classes of spatial distances (i.e. up to 2 km). In those two distance classes, the average estimated kinship coefficient values were significantly higher than the estimate expected under a random spatial distribution of genotypes. The slope of the correlation between kinship coefficient and the log spatial distance between pairs of isolates was -0.003 (SE: ± 0.001), and was significantly different from zero (*P*-value < 0.001), providing a *Sp* of 0.003.

3. Demographic history

We used an ABC approach to test which demographic scenario(s) best explained the data. The analysis showed that the group of scenarios that assumed an ancient reduction of the *A. ostoyae* population (i.e. SC2, 7 and 6, in decreasing order of probability), was selected with a higher posterior probability (61.5%; Table 2). The scenario 2, assumes an ancient reduction of a large *A. ostoyae* population (N_a) to reach its current effective size (N_1) and has the highest posterior probability value of 24.3% relative to the others, with a non-overlapping 95% confidence interval (23.4–25.3) (Figure 2; Table 2). The two others (SC7 and 6) received slightly lower posterior probabilities but higher than the other scenarios (21.4 and 15.8%, respectively).

Analysis of confidence in scenario choice revealed that only 32.2% of the pseudo-observed datasets simulated under the scenario 2 were correctly identified as having been generated under the scenario 2. This means that 67.8% were incorrectly identified as having been generated under

Scenario	Post. Prob. (95% CI)	P(Scenario 2)	Number of outlying summary statistics		
			$P < 0.05$	$P < 0.01$	$P < 0.001$
Scenario 1	13.5% (12.7– 14.3)	0.19‡	1	0	0
Scenario 2	24.3% (23.4–25.3)	0.32†	0	0	0
Scenario 3	5.5% (4.9–6.1)	0.05‡	1	0	0
Scenario 4	6.9% (6.2–7.5)	0.20‡	0	0	0
Scenario 5	6.1% (5.4–6.7)	0.03‡	1	0	0
Scenario 6	15.8% (14.9–16.6)	0.01‡	0	0	0
Scenario 7	21.4% (20.4–22.3)	0.02‡	0	0	0
Scenario 8	4.6% (4.0–5.2)	0.08‡	1	0	0
Scenario 9	2.1% (1.5–2.6)	0.02‡	1	0	0

Table 2: Model choice procedure and ABC performance analysis. Post. Prob.: relative posterior probability for each scenario. Details on the *P*-value calculation are provided in Materials and Methods and Table S3. Individual *P*-value reported for each summary statistic and each scenario are provided in Table S3. †: 1 - P(Scenario 2) represents, for the Scenario2, an empirical estimate of the type I error rate (here: 68%). ‡: P(Scenario 2) represents, for all scenarios except the Scenario 2, an empirical estimate of the scenario specific type II error rate.

other scenarios while they were in fact produced under the scenario 2 (i.e. type I or α error rate; Table 2). For the pseudo-observed simulated datasets using the eight other demographic scenarios, 7.5% on average were incorrectly identified as having been generated under the scenario 2 (i.e. type II or β error rate; Table 2), indicating a very good statistical power of our model choice procedure of 92.5% (i.e. 1- $\Sigma\beta_i$) to discriminate among the competing scenarios.

When comparing the values of summary statistics computed from simulated datasets for each of the nine scenarios against the observed values, the datasets simulated under the ancient decline scenarios (SC2, 6 and 7), were all compatible with the summary statistics of the observed data (Table 2 and Table S3). The posterior probability density parameters were estimated using the best demographic scenario (i.e. scenario 2; Figure S4). It suggest that a large *A. ostoyae* effective population of 6,820 individuals (95%CI: 1,580–9,840) was reduced to a current effective population size estimated to 672 individuals (95%CI: 555–4,500). The posterior probability of t_1 indicated that this decline would have occurred 1,700 generations BP (95%CI: 338–4,810). However, the scenario 7 received the second highest posterior probability value (Figure 2; Table 2), and suggests that the ancient reduction might have been followed by a more recent expansion. Marginal posterior distribution obtained from this scenario suggests that

the population expansion could have occurred very recently about 5 generations BP (*t*2: 95%CI: 2.60–9.76).

Discussion

We revealed a significant pattern of IBD at the spatial scale of a few kilometers that suggested that most of spore dispersal occurs over short distances via basidiospores. This dispersal is consistent with model of aerial spore dispersal in numerous fungal species proposed by Aylor (1989); long-distance spore dispersal at several hundred meters being possible but rare. This model is also consistent with epidemiological study of the disease suggested that pre-existing forests areas were the inoculum source for the colonisation of the neighbour newly planted forests (i.e. in a vicinity of 3 km; Labbé *et al.* 2015). As estimated at the scale of the massif in Prospero *et al.* (2008), no genetic structure was however observed at a larger spatial scale (i.e. at more than 3 km). This contrast between local and regional scales is regularly observed in population genetic studies (see for example Puebla *et al.* 2012). Rare long distances dispersal events may be sufficient to homogenize the genetic diversity at this spatial scale; classical genetic estimates such as F_{ST} being little powerful to infer long distance dispersal (Wingen *et al.* 2013). This result at this spatial scale of thousands hectares also suggested that gene flow (even low) was likely maintained among pre-existing forests fragments, at least between the warming period and the human settlement. Theoretical and experimental studies showed that only nearly absence of gene flow and long separation time among populations can create strong genetic differentiation, especially for large populations (e.g. Holderegger & Di Giulio 2010). Consequently, at the spatial scale studied here, we would not be able to identify different genetic sources having contributed to a possible expansion of *A. ostoyae* populations. However, we only sampled the west of the massif where pre-existing fragments were large and close among themselves. In the center of the massif where pre-existing forest areas were more scattered (Vallauri *et al.* 2012), new genetic analyses could estimate more differentiation among *A. ostoyae* populations. By contrast, results on the geographical distribution of allelic richness or Nei's genetic diversity more supported the colonization model of new planted areas by spores coming from the close pre-existing forest fragments. We identified a clear genetic gradient with pre-existing forest areas having more genetic diversity than new planted areas won on the marsh. This genetic gradient is in agreement with theoretical and experimental genetic results observed during population expansion, and associated with a decrease of genetic

diversity in the front of colonization due to new populations founded by a limited number of individuals (e.g. Excoffier *et al.* 2009). This genetic pattern observed on few kilometers is in agreement with a limited dispersal from a few neighbor sources, and the slow establishment of new disease foci in afforested areas for 150 years (Labbé *et al.* 2015).

The inference of the demographic history of the pathogen by the ABC approach suggests that the population of *A. ostoyae* had underwent a significant reduction (around 90%) of its effective size during an event that would have occurred probably more than one thousand of generations before today. Surprisingly, despite the recent population growth of the host, the scenario assuming both an old reduction and a recent expansion (around 60%) following this plantation was not the best scenario, but emerged clearly from other demographic scenarios. One possible explanation of this result could be due to an expansion not associated with a significant pathogen population growth relative to the population already present in the pre-existing forests. For example, only few genotypes, quickly producing fruiting bodies and many viable basidiospores may have contributed to this expansion, thus reducing the growth of *A. ostoyae* effective population size. The lowest genetic diversity observed in afforested areas, as well as the smallest number of isolated collected are in agreement to this explanation. A second explanation, taking into account the likely large effective time generation, assumed that the fungal expansion was probably difficult to detect with population genetic approaches such as ABC. It was already demonstrated that, because in very recent population size changes, the scale parameter ($\theta_0=4N_0\mu$) estimates at the sampling time converged to the real scale parameter during the ancient size ($\theta_1=4N_1\mu$), and thus could not detect a change in population size (Girod *et al.* 2011). In pathogen, such a genetic signature of recent expansion was observed for species with an annual sexual reproduction cycle (e.g. Barrès *et al.* 2012), but there is no study to date for root pathogen with long generation time (see below).

If we assume that the decline of the *A. ostoyae* population (1700 generations ago) observed in the ABC analysis may have occurred during the maximum cooling of the last glaciations (20,000 to 18,000 years BP), when the forest areas and therefore the hosts of the pathogen underwent the strongest reduction, an estimate of the generation time for *A. ostoyae* would roughly be 11 years (with an CI of 4 to 59). Considering the seventh scenario possible but difficult to accept (high error of type I), our results gave an estimated of the expansion of the pathogen occurring approximately five generations BP. If this

event corresponds to the beginning of the massive plantation in the Landes de Gascogne during the second half of the 19th century, the generation time of the pathogen would be estimated between 20 and 30 years. On the other hand, if this emergence occurred at the beginning of the intensification of the forestry (approximately in the second half of the twentieth century), the generation time would be estimate at approximately 10 years, very consistent with the first estimation. These two estimates for two different events give more support to the generation time of *A. ostoyae* between 10 and 20 years. In any case, this estimation of generation time must be taken with caution as those very recent expansions are extremely difficult to date precisely and especially with the very limited number of loci available for this study. Although the effective generation time for *Armillaria* species, as well as for other root rot pathogens is difficult to estimate, this result seems to be correct for isolates living in this south-western French forest. Although, some isolates of these species may live during many years (sometimes more than one thousand years, as estimated for *A. gallica* in North America; Smith *et al.* 1992), age-record are likely associated with extreme climatic conditions (cold and dry) and slow ecological dynamics of the boreal forests (Ferguson *et al.* 2003); very different that is observed at the study site. Out of the genotypes collected in this study, only two were part of the same clone and were only a hundred meters distant apart. These results confirmed that *A. ostoyae* disease foci rarely exceed one hectare in the Landes de Gascogne forest (Prospero *et al.* 2008), and reflect the greater dynamics of the planted forest compared to a natural one. Furthermore, age of the first sexual reproduction after the establishment of one genotype in a new disease center is likely variable among genotypes, but probably not higher than few tens of years. On one hand, some experimental infections on young pine seedlings in greenhouse showed rare productions of fruiting bodies few months after the beginning of the experiment (C. Dutech, pers. obs.). On the other hand, development of sub-cortical mycelium up to the collar of the infected tree, generally associated with the production of fruiting bodies (F. Labbé, pers. obs.), can be delayed from several years after infection when the host is resistant to this infection, especially at intermediate ages (i.e. 10-20 years-old; Lung-Escarmant & Guyon 2004). Variations in age of the first reproduction among isolates may also be increased by the low efficiency of sexual reproduction to produce a new genotype infecting a tree. It is commonly thought that germination of basidiospores, and fusion with another compatible haplotype are rare events, occurring under limited environmental conditions (Rishbeth 1988; Dettman & van der Kamp 2001). In

conclusion, this estimated age of 10-20 years is likely determined by the combination of first, the possibility for each genotype to produce fruiting bodies each fall, second, the large variation in time to produce this first fruiting body, that is dependent of the ability to rapidly colonize the root system of the host (i.e. from one to several years depending of the host age), and third the low success of establishment of progenies.

The effective population size of *A. ostoyae*, was estimated around 1,000 individuals (IC: 555–4,500), and may seem small compared to the large surface occupied by the maritime pine in the prospected area (approximately 20,000 ha); density will be less than one breeding individual per 10 ha. There is a small number of studies trying to estimate population size. Effective population size of the pathogenic fungus of wheat, *Mycosphaerella graminicola*, and of *Erysiphe graminis*, a pathogen of barley with both asexual and sexual reproduction, was estimated, using the Ewens sampling formula (Ewens 1972; Nei & Tajima 1981), at approximately 24,000 and at 4.4×10^{9}, respectively (Damgaard & Giese 1996; Zhan & McDonald 2004). Similarly, the effective size of the mycorrhizal fungi *Claroideoglomus etunicatum*, was estimated at more than 400,000 individuals (VanKuren *et al.* 2013). Our results are more consistent with the effective population size of another the fungal pathogen *Puccinia striiformis* that causes wheat yellow/stripe rust, which was estimated using temporally spaced samples at approximately 1700 individuals (Ali 2013). This effective population size thus also reflects the limited sexual reproduction capabilities of *A. ostoyae* and that despite a significant amount of disease foci, only a small proportion of individuals contributing to the next generation.

In conclusion, although possible, the recent expansion of *A. ostoyae* using population genetic method was not clearly identified. A study at larger scale including more isolates from the central part of the massif and additional genetic markers to improve the power of analysis is a perspective to better conclude on the history of *A. ostoyae*. Another possibility may be a monitoring of the disease during several years close to the pre-existing forest areas, as identified in Labbé *et al.* (2015). Because previous monitoring during 30 years by the DSF is only based on large mortality (i.e. several tens of square meters), it could be biased if most of new established disease foci are first associated with low mortality (few trees) within stands. By systematic annual monitoring, we can expect to observe new disease centers year after year in the margins of these pre-existing forests. Assuming the effective dispersal of basidiospores in the range of few

kilometers, and in agreement with the IBD pattern identified in this study, the occurrence of these new disease centers should decrease with the spatial distance from these borders. Such a monitoring would be interesting, on one hand to better understand the exact demographic dynamics of *A. ostoyae*, but on the other hand, to better predict, relative to the geographical locations in the massif, the risk associated to this pathogen.

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Supporting information

Locus		Primer sequences (5'-3')	Motif	Size	[C]	Dye
Multiplex						
AoB8A4Z	F:	GAGAATGCGGAAGGAAGAGA	$(AGG)_n$	74-107	0.19	PET
	R:	CTCGTCTGAAGGAGTGAATGG				
AoB9MK4	F:	GGTGATAGGTCAGCAGTAGCG	$(AG)_n$	85-87	0.13	VIC
	R:	ACGACTGGTCTTTCTGTCCG				
AoCE9NK	F:	CGTGAAGTCGTAAAGCGTCG	$(AGC)_n$	64-76	0.19	FAM
	R:	AAGTCTCTGAGATCGCCTCAT				
AoSSR21a	F:	GCAGAGCGCAAATGAAACTA	$(CA)_n$	92-124	0.25	NED
	R:	CACCACGAGTGCTTCTACCA				
AoSSR75a	F:	GTAATCGTCCACTGCTCGG	$(GT)_n$	128-	0.19	VIC
	R:	CATCAACTCAAAACCCTTGC				
Arm05	F:	GAGGAAGAGCTACGCACAGG	$(GTC)_n$	226-	0.19	FAM
	R:	CGGTTTCATCGGAGGTCTA				
Arm15	F:	CGAGCCGTCAACAGAGAATC	$(GAC)_n$	175-	0.13	NED
	R:	TCCCCAAACACAACCTTCTC				
Multiplex						
AoB8PN1	F:	CAGCCTAGCGTGAACAACAC	$(AC)_n$	84-87	0.13	VIC
	R:	GTTGTTGCTCCCTTCCACAC				
AoCFZOL	F:	CACTCTAAATCTCTACTCAATCCCA	$(AC)_n$	68-72	0.13	NED
	R:	AATTCCTTGCTCCCTTCCAC				
AoSSR74a	F:	GCTCACCCTCAAACTTAACA	$(GT)_n$	95-102	0.25	FAM
	R:	GCAGGGCACAAATGAAACTA				
Arm09	F:	CGTCTCTGGTCCATGAAGGT	$(GTT)_n(CTG)_n$	178-	0.13	PET
	R:	GCCTCAGCAGCACCAGAT				
Arm16	F:	ATTTGGAATCCTGACGTTGC	$(TCG)_n$	137-	0.13	VIC
	R:	GGCGCATTTGGTCAAAGTAA				
CAG25a	F:	AGGACTTCCAGAGGATGATGA	$(CAG)_n$	235-	0.19	VIC
	R:	TATGACCCACCACCACCTG				
CAG77a	F:	TAGCCTGCGGTTACGATGAC	$(CAG)_n$	231-	0.25	NED
	R:	AGTGGCTCCTCAATCTTTGG				

Tables S1: Characteristics of the two multiplexes. [C]: primer concentration in the primer premix [µM].

Priors for the demographic parameters	
N_1	UN ~ [500 – 5000]
N_a	UN ~ [1000 – 10000]
N_b	UN ~ [10 – 1000]
t_1	UN ~ [100 – 5000]
t_2	UN ~ [1 – 100]
Constraint on parameters	$t_2 < t_1$; $N_1 < N_a$; $N_1 > N_b$

Priors for the mutation model	
MEAN - μ	UN ~ [1 x 10^{-4} – 1 x 10^{-3}]
GAM - μ	GA ~ [1 x 10^{-5} – 1 x 10^{-2}, 2]
MEAN - *P*	UN ~ [0.1 – 0.3]
GAM - *P*	GA ~ [1 x 10^{-2} – 9 x 10^{-1}, 2]
MEAN - *SNI*	LU ~ [1 x 10^{-8} – 1 x 10^{-5}]
GAM - *SNI*	GA ~ [1 x 10^{-9} – 1 x 10^{-4}, 2]

Table S2: Priors for the ABC. Uniform distribution (UN) with 2 parameters: min and max; Gamma distribution (GAM) with 4 parameters: min, max, shape; Log-Uniform (LU) distribution with 2 parameters: min and max.

	Summary statistics (*S*)			
	A	H_e	*V*	M_{GW}
Obs. *S*	5.86	0.57	4.57	0.68
	Prob. ($S_{simul.} < Q_{obs.}$)			
Scenario 1	0.501	0.502	0.791	0.047*
Scenario 2	0.493	0.449	0.609	0.159
Scenario 3	0.548	0.566	0.871	0.045*
Scenario 4	0.416	0.312	0.681	0.095
Scenario 5	0.596	0.713	0.888	0.050*
Scenario 6	0.556	0.614	0.762	0.086
Scenario 7	0.513	0.584	0.588	0.195
Scenario 8	0.386	0.411	0.797	0.048*
Scenario 9	0.509	0.512	0.856	0.041*

Table S3: Model checking procedure. Prob. ($S_{simul.} < S_{obs.}$) Corresponding tail-area probabilities (*P*-values) were obtained as Prob. ($S_{simul.} < S_{obs.}$) and 1.0 - Prob. ($S_{simul.} < S_{obs.}$) for Prob. ($S_{simul.} < S_{obs.}$) 0.5 and > 0.5, respectively. We used the mean number of alleles per locus (*A*), the mean expected heterozygosity (H_e), the mean allele size variance (*V*), and the mean M_{GW} index across loci. *: $P \leq 0.05$; ** ≤ 0.01; *** < 0.001. Demographic scenarios from 1 to 9 are represented in Figure 2.

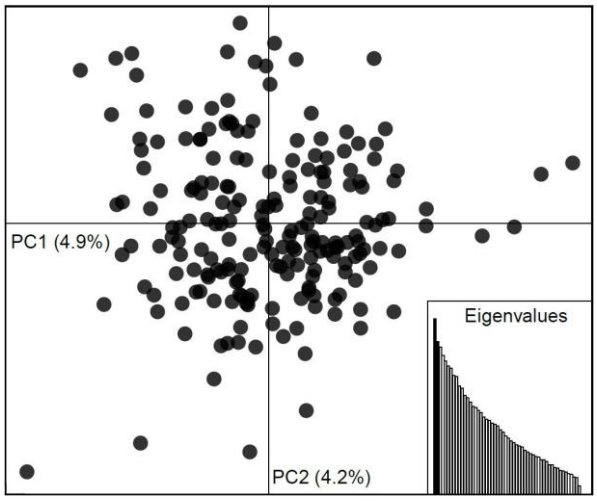

Figure S1: PCA of the *A. ostoyae* samples. Individuals are plotted according to their coordinates on the first two principal components (PC1 and PC2 respectively). The black dots indicated the multilocus genotypes. Eigenvalues corresponding to the represented components are filled in black.

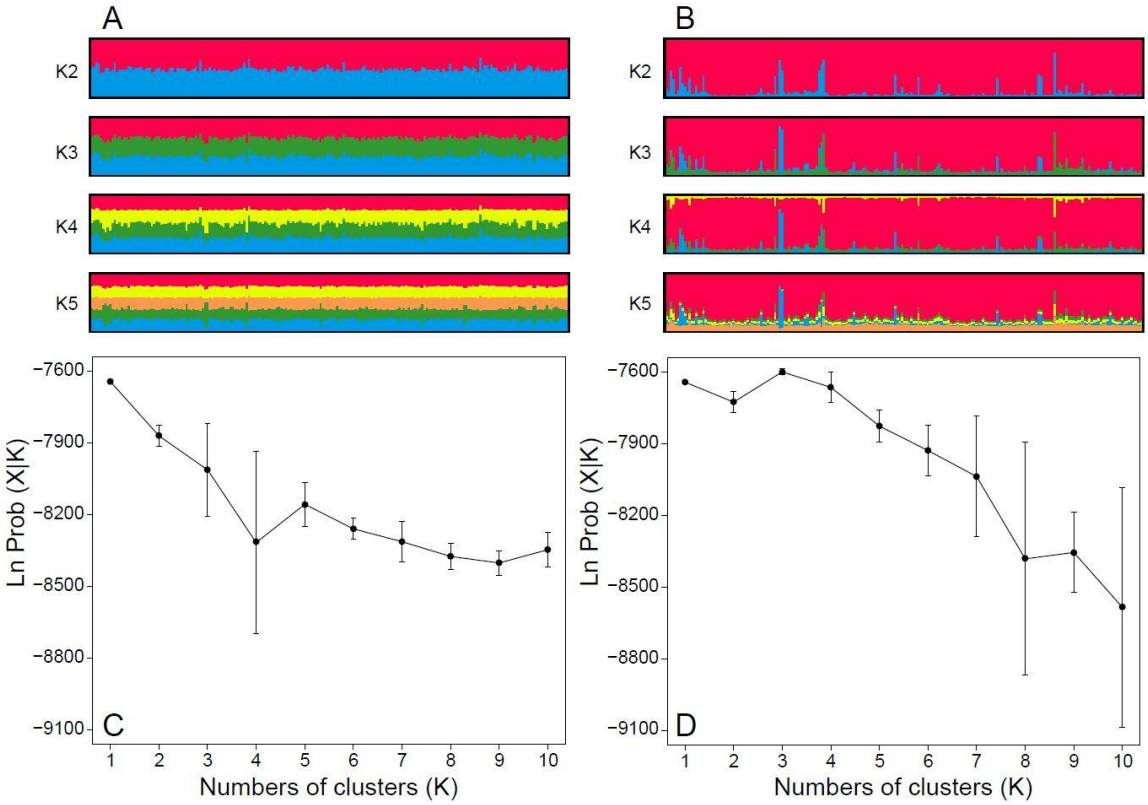

Figure S2: STRUCTURE bar plots for each value of *K* from 2 to 5 generated from microsatellite and SNP data for standard admixture model (A) and *locprior* admixture model (B). Each vertical line represents an individual, and the color composition displays the probability of belonging to each of the clusters. The probability of observing the data (*X*) under a given number of putative clusters (*K*) is shown as a function of *K*, for the standard model (C) and the *locprior* model (D). The error bars provide the standard deviation observed over 10 replicated runs for each value of *K*.

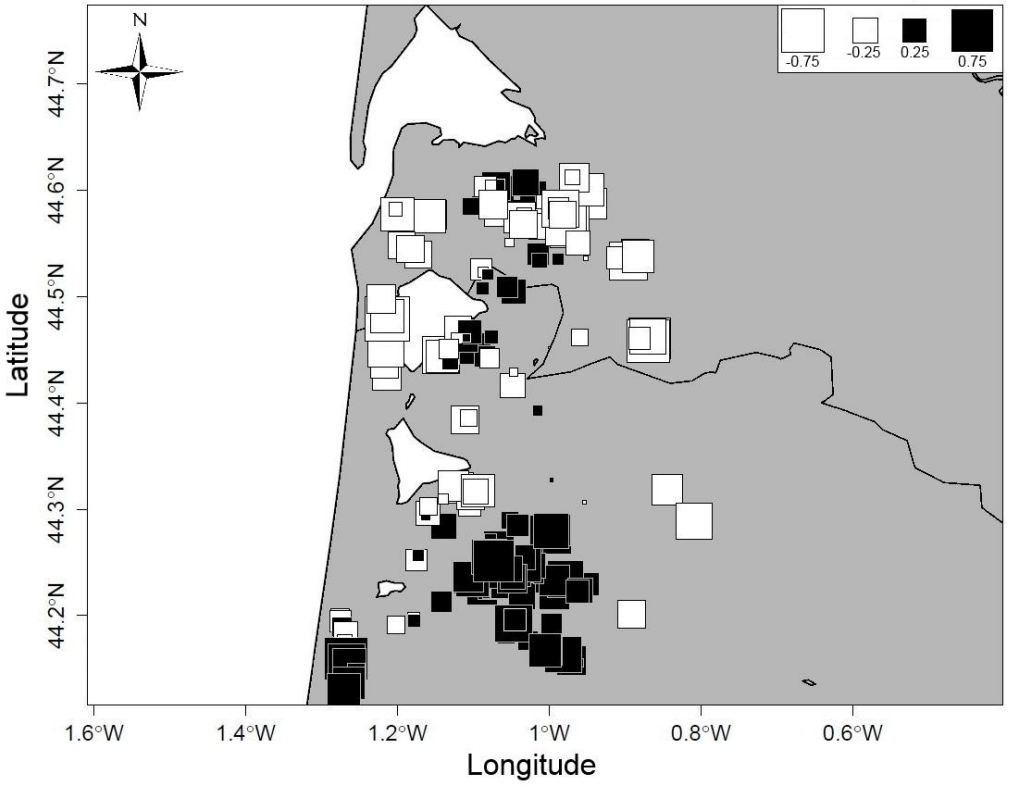

Figure S3: Graphical display of the global spatial genetic structure of *A. ostoyae* detected by sPCA. Each *A. ostoyae* sample is represented by a square. The black squares correspond to the positive sPCA scores, and the white squares to the negative sPCA scores. The size of the squares is proportional to the absolute value of the sPCA scores.

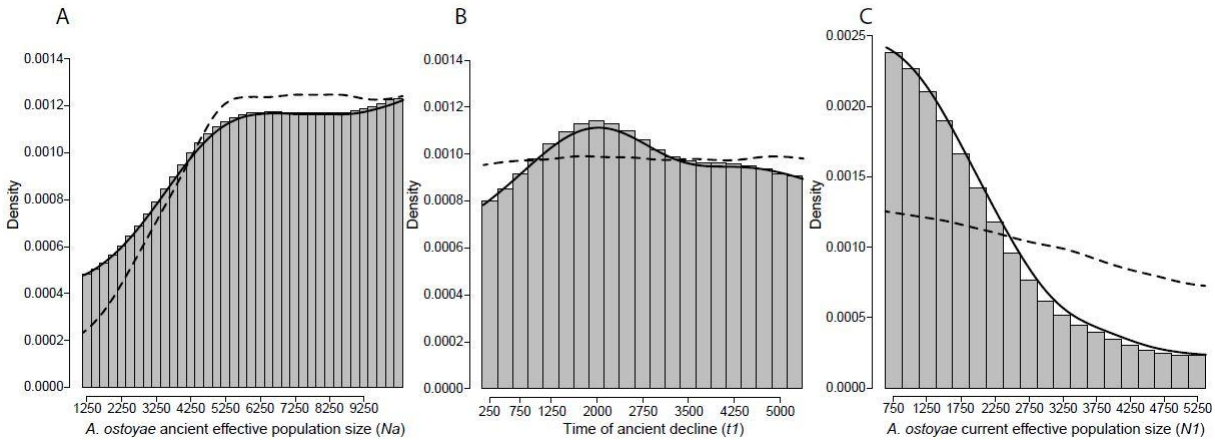

Figure S4: The posterior probability density (histograms and solid lines) estimated under the best demographic scenario (i.e. scenario 2). A: ancestral population effective size (N_a); B: timing of the ancient reduction of the population effective size (t_1); C: current population effective size (N_1). The dotted lines show the prior distributions for each parameter.

CHAPITRE V

Discussion générale

I. Cycle biologique d'*A. ostoyae* dans le massif landais

Bien que certaines zones d'ombre persistent, l'approche multidisciplinaire (épidémiologie, pathologie et génétique) utilisée dans cette thèse nous permet au terme de ce travail d'éclaircir un certain nombre de processus mal connus du cycle biologique d'*A. ostoyae*, grâce à la caractérisation plus précise des traits impliqués dans sa phase parasitaire, de la variabilité de la capacité saprophytique, et de l'efficacité de ses différents modes de dispersion dans le massif forestier des Landes de Gascogne.

1. Traits impliqués dans la phase parasitaire

La corrélation positive et significative observée entre la rhizomorphogénèse et la pathogénicité d'*A. ostoyae* lors des premiers mois d'inoculation artificielle en serre, confirme que la production de rhizomorphes est fortement impliquée dans la pathogénicité du parasite comme observé dans d'autres populations. Dans l'expérience menée, cette production de rhizomorphes facilite probablement la rencontre avec les racines des semis, bien que le protocole de l'expérimentation choisie dans la thèse devait minimiser cet effet en collant l'inoculum au plus près des racines. Cette capacité est peut-être aussi associée à la faculté de coloniser rapidement les tissus racinaires. L'implication des rhizomorphes dans la virulence des isolats est cependant difficile à relier aux mortalités observées dans les Landes, du fait du peu de rhizomorphes observés dans les plantations de pin maritime du massif forestier (Guillaumin & Legrand 2005). La propagation d'hôte en hôte par les contacts racinaires reste l'hypothèse la plus probable dans ce contexte forestier particulier, où la densité d'hôtes est importante. Cependant, ce rapprochement des systèmes racinaires, qui n'implique pas forcément l'établissement de contacts directs des racines, facilite également les infections par les rhizomorphes. Par conséquent, il n'est pas nécessaire que la production de rhizomorphes soit importante *in situ* mais simplement rapide pour qu'*A. ostoyae* puisse infecter son hôte. Même si les rhizomorphes ne constituent pas la principale voie d'infection d'*A. ostoyae* dans les Landes de Gascogne, les isolats aux meilleures capacités de rhizomorphogène pourraient malgré tout infecter plus facilement les pins dans le massif.

Finalement, bien que la production de rhizomorphes contribue à la virulence d'*A. ostoyae*, l'atténuation après quatre mois, de la corrélation entre la production de rhizomorphe et la virulence des isolats suggère l'existence d'autres processus. Cependant, il est difficile de dire à partir de nos résultats comment la phase de progression du parasite dans le cambium est impliquée dans la virulence d'*A. ostoyae* dans les Landes. En effet, la méthode d'inoculation,

employée afin d'évaluer la capacité du pathogène à provoquer des lésions, n'a pas permis de différencier significativement les isolats pour cette aptitude. Il est donc envisageable que cette aptitude soit identique pour les isolats d'*A. ostoyae,* ou bien plus probablement que cette observation résulte des limites de la méthode d'inoculation utilisée. Il semble donc nécessaire de développer une nouvelle méthode pour évaluer la variabilité de ce trait. Des inoculations de branches de jeunes arbres sur pied, sans blessure préalable, c'est-à-dire par l'accolement de l'inoculum sur l'écorce, permettraient dans un premier temps d'accroître le nombre de répétitions, mais aussi de se rapprocher des conditions d'infection naturelle. En effet, la blessure des branches, bien qu'initialement effectuée pour faciliter l'infection en rapprochant au maximum l'inoculum du cambium, provoque un écoulement de résine et un début de cicatrisation (Solla *et al.* 2002) qui pourraient à l'inverse accroître la difficulté d'infection par *A. ostoyae*. L'association de cette méthode d'inoculation sur tiges vivantes non blessées avec les récentes méthodes moléculaires de détection spécifique du genre *Armillaria* à partir de faibles quantités d'ADN du pathogène dans le bois (Gonthier *et al.* 2015), permettrait également par plusieurs points de sondages autour du site d'inoculation, de conforter la présence du pathogène dans les zones lésées, et donc, d'évaluer plus rigoureusement la taille des surfaces colonisées par *A. ostoyae* sans passer par une mesure visuelle des zones nécrosées, sujette à erreur, comme réalisée dans le chapitre III.

Enfin, bien que nous permettant de rapidement évaluer la virulence des isolats d'*A. ostoyae*, l'utilisation de pins maritimes de deux ans, particulièrement sensibles à cet agent pathogène, ne nous permet pas de généraliser aux arbres plus âgés les profils d'agressivité observés sur ces jeunes plants. En effet, la mise en place de mécanismes de défenses plus efficaces chez les arbres adultes pourrait être responsable de changement dans les mécanismes d'infections. Par conséquent, il est possible que les isolats les plus agressifs sur jeunes pins ne correspondent pas nécessairement aux isolats les plus agressifs sur les plus vieux. De plus, ces inoculations de pin maritime ont uniquement été réalisées en serre, or Omdal *et al.* (1995) ont, par exemple, montré des différences importantes de sensibilité du sapin blanc (*Abies alba*) en serre et en forêt. Ces variations de sensibilité peuvent résulter de problèmes biotiques et abiotiques rencontrés par l'arbre en milieu naturel, mais rares ou absents en serre. Des inoculations parallèles d'un même isolat d'*A. ostoyae* sur des hôtes d'âges différents, bien que techniquement difficiles à mettre en place et risquées d'un point de vue de la possible dispersion de la maladie sur le terrain, permettraient de valider les résultats acquis sur jeunes plants.

2. Trait impliqués dans le comportement saprophyte

Les faibles capacités de dégradation du bois mort mises en évidence pour les isolats étudiés sont en accord avec celles décrites par les études de Guillaumin & Lung (1985), Robene-Soustrade (1993) et Prospero *et al.* (2004). Ce faible pouvoir saprophyte, en comparaison de nombreux autres champignons lignivores (Tanesaka *et al.* 1993), suppose qu'en l'absence d'une pression d'inoculum importante, *A. ostoyae*, serait donc peu compétitif face à ces autres espèces décomposeurs du bois, ne coloniserait alors que très difficilement les substrats ligneux du sol, sauf sous certaines conditions particulières. Une des ces conditions est une forte humidité des substrats. Le développement d'*A. ostoyae* sur les grumes de bois stockés sous aspersion, mis en place suite à la tempête Klaus de 2009, illustre, s'il le fallait, l'effet de ces conditions humides sur le développement saprophytique du champignon. Alors que l'engorgement en eau protège le bois d'un grand nombre d'insectes (*Ips sexdentatus* par exemple) et de champignons saprophytes ou responsables du bleuissement du bois (*Ceratocystis* sp. par exemple), ces conditions sont en revanche très favorables à l'Armillaire. *A. ostoyae* bénéficierait également d'un avantage par rapport aux autres lignivores lorsqu'le champignon est le premier à coloniser la niche écologique que représente le bois mort. La présence initiale du pathogène dans des lésions latentes dans le système racinaire de son hôte lui fournit alors un avantage certain par rapport aux autres champignons saprophytes, même si leur capacité de dégradation est jusqu'à cinq fois supérieure à celle de l'Armillaire. La lente décomposition du bois qui s'en suit, ainsi que la mise en place par le pathogène de "zone-lines" (Campbell 1934; Lopez-Real 1975) qui le protège contre les champignons antagonistes, permettrait alors au pathogène de persister dans le bois mort colonisé pendant plusieurs dizaines d'années, comme cela a été notamment démontré sur *A. mellea* (Rishbeth 1972). Il semble donc que cette phase saprophyte d'*A. ostoyae* ne constitue qu'une phase de transition lui permettant de se maintenir suffisamment longtemps pour augmenter ses chances d'infecter un nouvel hôte. En conditions naturelles, l'état saprophytique d'*A. ostoyae* serait donc la conséquence de son parasitisme

L'absence de relation entre cette phase saprophytique et la production de rhizomorphes, exclue l'hypothèse d'une meilleure propagation d'*A. ostoyae* dans le sol pour les isolats les plus saprophytes. En revanche, la relation entre les capacités de dégradation du bois d'un isolat d'*A. ostoyae* et sa capacité à se maintenir longtemps dans le bois mort n'a jamais été évaluée et constitue une hypothèse intéressante. Des expériences d'isolements sur plusieurs années à partir de souches colonisées par l'Armillaire obscure couplées à des évaluations de l'activité lignivore des isolats collectés sur les souches, permettraient de tester cette relation

entre la dégradation du bois et la conservation dans le sol. Comme déterminé dans le chapitre III, une rapide estimation de la capacité saprophytique des souches peut-être réalisée à partir de mesures de croissance mycélienne in vitro. La simplicité de cette méthode permettrait de tester les capacités saprophytiques d'une grande quantité d'isolats issus de l'ensemble du massif forestier et donc d'étudier plus facilement les gènes impliqués, leur diversité et leur répartition géographique dans cette phase du cycle d'*A. ostoyae*. Toutefois, bien que faible, cette capacité est variable dans le massif des Landes. Ces variations importantes de la phase saprophytique du pathogène semble témoigner de stratégies évolutives différentes pour des isolats d'une même espèce d'Armillaire au sein d'un même écosystème.

3. Modes de dispersion

a. La croissance végétative

Bien qu'il soit difficile à partir de mon étude d'évaluer précisément l'étendue des surfaces occupées par les génotypes d'*A. ostoyae*, notamment en raison de l'échantillonnage qui se focalise sur des foyers distants au minimum d'une centaine de mètres, l'estimation de la surface d'occupation moyenne d'un génotype d'*A. ostoyae in situ*, et l'importante production de rhizomorphes quantifiée en laboratoire, suggèrent que la croissance végétative du pathogène joue potentiellement un rôle majeur dans la dispersion d'*A. ostoyae* à courte distance. En effet, seuls deux isolats parmi 220 ont été identifiés comme appartenant à un seul et même génotype et associés à une dispersion clonale d'arbre en arbre. En supposant que ces deux échantillons, soient localisés aux extrémités du diamètre d'un rond de mortalité, alors ce génotype occuperait une surface d'environ deux hectares. Cette surface est dans la gamme de celles estimées par les précédents travaux réalisés par Prospero *et al.* (2008) et Lung Escarmant & Taris (1984) dans les Landes de Gascogne, qui rapportent des surfaces excédant rarement quelques hectares. Ces surfaces sont légèrement supérieures à celles mesurées dans les forêts mixtes d'Auvergne dans le centre de la France (Legrand *et al.* 1996). Il est fort probable que la propagation d'hôte en hôte de l'Armillaire, par les rhizomorphes ou les contacts racinaires, soit facilitée par la forte densité de pins maritimes dans les Landes de Gascogne, dont le nombre de tiges évolue entre 1100 tiges à l'hectare lors de la plantation, et 300 tiges à l'hectare avant la récolte finale. Une telle densité réduit l'espace entre chaque plant à trois mètres seulement en début de plantation, ce qui augmente les probabilités de contacts entre les racines d'hôtes voisins, et de contamination par l'Armillaire via des palmettes lors de contacts racinaires entre un arbre infecté et un arbre sain voisin (Shaw 1980; Lung-Escarmant *et al.* 2003), ou plus rarement via ses rhizomorphes (Zeller 1926 et Childs &

Zeller 1929). Toutefois, l'étendue des génotypes d'*A. ostoyae* dans les Landes de Gascogne, diffère fortement de celle en Colombie-Britannique pour laquelle Ferguson *et al.* (2003) ont observé un génotype pouvant atteindre jusqu'à 1000 hectares environ. Il est surprenant de constater que les génotypes d'*A. ostoyae* occupent de si faibles surfaces alors que les plantations monospécifiques de pins maritimes par leur forte densité et leur importante sensibilité à *A. ostoyae*, peuvent grandement faciliter la propagation de l'agent pathogène. Il peut être envisagé qu'entre deux plantations successives (environ deux ans), la surface occupée par *A. ostoyae* diminue par réduction des ressources ligneuses et donc réduction de l'inoculum contenu dans les plus petits fragments ligneux du sol. Des expériences de suivis de l'évolution de foyers de la maladie entre deux rotations de peuplement et pour des conditions différentes de gestions forestières permettraient de tester cette hypothèse.

b. Etablissement de nouveaux foyers par les basidiospores

La question de l'efficacité de la dispersion des basidiospores issues de la reproduction sexuée a été discutée dans de nombreuses études (voir par exemple Prospero *et al.* (2008). Cette thèse révèle par plusieurs aspects que ces basidiospores contribuent bien à la dispersion de la maladie, et notamment à l'installation des nouveaux foyers : l'absence de sous-structures génétiques identifiables à l'échelle de cette étude, d'écarts aux attendus de Hardy-Weinberg et de fort taux de clonalité sauf localement, sont des indices allant dans ce sens. Cette dispersion se fait probablement majoritairement sur quelques kilomètres comme le suggère l'agrégation spatiale des foyers de la maladie, ainsi que le patron d'isolement par la distance observé sur cette gamme de distance. Prospero *et al.* (2008) n'avaient pas pu mettre en évidence un patron d'isolement par la distance et concluaient donc à une forte implication de la dissémination par le vent des basidiospores dans la propagation d'*A. ostoyae*. Toutefois, il est fort probable que leur échantillonnage, alors basé sur une trentaine de foyers de seulement quelques hectares à l'échelle du massif forestier, soit à l'origine de l'absence d'identification d'un isolement par la distance. Ces distances de dispersions d'*A. ostoyae* sont de plus similaires à celles du fomès (*Heterobasidion annosum*), un autre agent de pourridié à l'origine également d'importantes mortalités de conifères (Möykkynen *et al.* 1997). Des expériences de fructification *in vitro* (Korhonen 1980; Guillaumin 1986), couplées à des piégeages et des quantifications de spores *in situ*, permettraient d'estimer l'aptitude d'*A. ostoyae* à se disperser à longue distance pour différents génotypes. Des expériences *in situ* complémentaires d'évaluation des taux de germination de différentes concentrations de basidiospores d'*A. ostoyae* sur bois mort (souches et fragments ligneux par exemple), comme cela l'a été entrepris sur *A. novae-zelandiae* en Nouvelle-Zélande (Hood *et al.* 2008), seraient également nécessaires afin de

différencier la dispersion théorique de la dispersion efficace du pathogène en conditions naturelles. De telles expériences permettraient également d'identifier les sites où s'effectuent de préférence les germinations des basidiospores actuellement très mal connus pour *A. ostoyae*.

4. Temps de génération

Le cycle de vie pérenne des espèces appartenant au genre *Armillaria*, qui leur permet notamment dans certains cas de présenter de très longues durées de vie (Smith *et al.* 1992; Ferguson *et al.* 2003), complique fortement l'estimation du temps de générations de ces champignons, c'est-à-dire du temps moyen nécessaire à un individu pour contribuer à la génération suivante. Bien que les espèces d'Armillaires présentent un fort impact dans les forêts du monde entier (Laflamme & Guillaumin 2005), jusqu'à maintenant aucune étude n'avait estimé le temps de génération de l'une de celles-ci, ou même d'un autre agent de pourridié. Selon l'approche ABC, les scénarios démographiques les plus pertinents témoignent d'une réduction de la population d'Armillaire dans les Landes de Gascogne qui se serait réalisée il y a environ 1700 générations avant notre ère, ainsi que d'une récente expansion qui aurait eu lieu il y a seulement 5 générations. Les baisses importantes des températures au cours du maximum glaciaire de la dernière glaciation (-20000 et -18000 ans), ainsi que les plantations massives de pins maritimes initiées en 1857, correspondent probablement aux événements anciens et récents ayant respectivement le plus marqués l'histoire démographique de la forêt dans le sud-ouest de la France, et donc probablement du pathogène infectant essentiellement les résineux. En conséquence, si l'on considère que l'ancienne réduction et la récente expansion de la taille efficace de la population d'*A. ostoyae*, se sont respectivement déroulées lors de ces deux changements démographiques majeurs de la forêt, alors on peut estimer le temps de générations d'*A. ostoyae* à une dizaine d'années environ. Ce résultat semble cohérent aves les connaissances actuelles du cycle biologique d'*A. ostoyae*. En effet, la formation des fructifications, à l'origine de la production des basidiospores, dépend fortement des conditions climatiques (froides et humides) et peut, en conséquence, ne pas avoir lieu certaines années, et donc, contribuer au rallongement du temps de génération. De plus, les nouvelles infections racinaires peuvent rester latentes plusieurs années avant de contourner les défenses de l'hôte et de pouvoir coloniser les racines traçantes ou le collet d'où émergent les fructifications. Cela doit être tout particulièrement marqué chez les hôtes entre 10 et 20 ans dont la résistance au pathogène est supérieure à celle des jeunes arbres (Lung-Escarmant & Guyon 2004).

II. Les stratégies évolutives d'*A. ostoyae* dans les Landes de Gascogne

Parmi les nombreuses espèces du genre *Armillaria*, *A. ostoyae* semble celle pouvant le mieux adapter sa stratégie selon l'hôte et les conditions environnementales (Guillaumin & Legrand 2005). Mon étude nous permet aujourd'hui de clarifier la stratégie adoptée par *A. ostoyae* dans le contexte particulier de l'une des plus grandes forêts de plantations monospécifiques de résineux d'Europe.

1. Parasitisme primaire et/ou secondaire ?

Tous les isolats d'*A. ostoyae* inoculés artificiellement en serre ont eu la capacité d'infecter et de tuer de jeunes pins maritimes en bonne santé. Ces isolats présentaient de plus une importante pathogénicité vis-à-vis du pin maritime, avec des taux de mortalité compris entre 50% et presque 95% en seulement six mois. Ces résultats contrastent avec d'autres études évaluant la pathogénicité de différents isolats d'*A. ostoyae*, qui soulignaient régulièrement la présence d'isolats n'ayant qu'une très faible agressivité pour son hôte (Prospero *et al.* 2004), ou même parfois ne pouvant infecter son hôte (Omdal *et al.* 1995). Il semble donc tous les isolats d'*A. ostoyae* testés puissent bel et bien se comporter comme parasite primaire du pin maritime, capable donc d'infecter et de tuer cette espèce même s'il elle ne présente aucun affaiblissement. Il faut cependant souligner que tous les isolats ont été prélevés sur des arbres fraîchement morts, et peut être plus fortement pathogènes par rapport à des isolats peut-être plus saprophytes. L'absence de corrélation entre capacité saprophyte et virulence sur jeunes pins ne plaide cependant pas pour cette hypothèse. Les prospections effectuées lors de mon étude ne nous permettent malheureusement pas de directement déterminer le comportement primaire ou secondaire d'*A. ostoyae* dans le massif forestier des Landes de Gascogne. En effet, il n'est pas rare que d'autres bioagresseurs (*Heterobasidion annosum* et *Ips sexdentatus* par exemple), aient également été observés sur les mêmes mortalités que l'Armillaire. La détermination du rôle joué par chacun d'eux vis-à-vis de la mort de l'arbre est par conséquent complexe : l'Armillaire est-il arrivé en premier et l'affaiblissement de l'arbre qui s'en suit aurait-il attiré d'autres ravageurs, ou est-ce l'inverse ? Seul des suivis dans le temps permettraient de répondre à cette question. De plus, un certain nombre de mortalités où la présence de l'Armillaire est observée, résulte possiblement de l'affaiblissement de l'arbre par des blessures mécaniques d'engins forestiers, des élagages. Il est, par conséquent, difficile d'attribuer un comportement précis de l'ensemble de la population d'Armillaire dans le massif forestier des Landes de Gascogne sur les arbres adultes. Par exemple, un isolat peu agressif

n'ayant pas les composantes d'agressivités suffisantes pour contourner les défenses de son hôte lorsqu'il est sain, pourrait donc uniquement se comporter en tant que parasite secondaire. A l'inverse, un isolat fortement agressif se comporterait comme un parasite primaire pouvant infecter et rapidement coloniser le système racinaire de son hôte même si celui-ci met en place d'importants mécanismes de défense.

Les résultats de ce travail indiquent donc de forts niveaux de virulence des isolats d'*A. ostoyae* des Landes sur le pin maritime. Ces résultats pourraient s'expliquer soit par une adaptation des isolats aux pins landais, soit par une sensibilité forte du pin maritime. En raison de la co-existence d'*A. ostoyae* et du pin maritime depuis plusieurs millions d'années dans le sud-ouest de la France, les isolats d'*A. ostoyae* pourraient en effet présenter une meilleure adaptation à leur hôte, et donc, une plus forte pathogénicité que d'autres isolats de la même espèce provenant de forêts où le pin maritime est absent. Des inoculations artificielles de deux isolats d'*A. ostoyae* provenant de forêts naturelles d'épicéa commun de Suisse (C2 et C18 dans Prospero *et al.* 2004) ont été menées en parallèle des premières inoculations des souches landaises, et ne soutiennent pas cette hypothèse. En effet, ces isolats ont la capacité d'infecter et de tuer de jeunes pins maritimes en conditions contrôlées dans la même gamme que celle mesurée pour les souches landaises. De plus, bien que ces isolats ne fassent pas partie des plus agressifs sur pin maritime, ils semblent toutefois conserver les mêmes profils que ceux décrits sur l'épicéa commun par Prospero *et al.* (2004) : C2 et C18 engendrant respectivement de faible et de fort taux de mortalité. Toutefois, une comparaison d'un plus grand nombre d'isolats de ces deux populations d'*A. ostoyae* permettrait de tester d'une manière plus robuste ces résultats. Les conclusions provisoires suggèrent qu'il s'agit plus vraisemblablement d'une plus forte sensibilité du pin maritime qui conduit à observer les fortes mortalités dans la forêt landaise, exacerbées peut-être par un contexte environnemental lié aux plantations très favorable à *A. ostoyae.* Cette étude est en accord avec une différence non significative de la virulence moyenne entre deux populations d' *A. ostoyae* observée en Colombie-Britannique (Morrison & Pellow 2002).

Cette étude montre également une forte variabilité de la virulence entre isolats d'*A. ostoyae* landais. Par conséquent, si les isolats les plus virulents sont sélectionnés, alors la virulence globale d'*A. ostoyae* à l'échelle du massif forestier pourrait progressivement augmenter, constituant une menace aggravée pour les futures plantations de pins. Dans le cadre de ce travail, je suis parti de l'hypothèse que les isolats d'*A. ostoyae* présentant les plus fortes pathogénicités vis-à-vis du pin maritime, sont ceux qui ont pu coloniser le plus rapidement les nouvelles régions boisées. Sous cette hypothèse, il était donc attendu une virulence accrue

chez les isolats échantillonnés dans ces nouvelles aires de plantation. Cependant, cette hypothèse n'est pas soutenue par les expériences d'inoculations artificielles qui ne mettent pas en évidence une pathogénicité plus marquée des isolats d'Armillaire obscure prélevés dans l'aire d'expansion par rapport à ceux prélevés dans l'aire de distribution ancienne (annexe du chapitre III). Il est possible que le nombre d'isolats comparés ne soit pas suffisant pour tester cette hypothèse. Cependant, la variation de la virulence à l'échelle du massif forestier pourrait simplement résulter du hasard. En effet, la dérive génétique, qui modifie plus fortement les fréquences alléliques que la sélection naturelle dans les petites populations (Fisher 1930), aurait pu agir fortement sur les petites populations d'*A. ostoyae* qui existaient dans les petites et fragmentées forêts mixtes précédant les plantations massives (Vallauri *et al.* 2012). Toutefois, la sélection pourrait s'effectuer actuellement et pourrait aboutir à la fixation d'un niveau de virulence accru de l'agent pathogène en relation avec le degré de transmission de l'agent pathogène dans la forêt actuelle (Alizon & Baalen 2005). Il est important de souligner aussi que l'expérience d'inoculation artificielle employée ne teste que des isolats collectés à proximité du littoral atlantique où la densité des forêts préexistantes, et donc de la maladie, est importante. Cette zone géographique n'est peut-être pas la meilleure pour tester deux populations contrastées puisque les nouvelles zones plantées sont souvent très proches des zones où préexistaient la forêt. Il peut donc y avoir des migrations compliquées entre les deux compartiments qui brouillent l'affectation des génotypes à l'une ou l'autre des catégories de zones forestières. Par conséquent, étendre l'expérience à l'échelle du massif forestier entier, en comparant cette fois des isolats collectés à proximité du littoral avec des isolats du plateau landais où la densité de ces forêts est nettement plus réduite, permettrait peut-être de tester dans de meilleures conditions cette hypothèse. Alternativement, il est possible que les isolats, ayant colonisés les nouvelles plantations de pins, correspondent finalement aux isolats ayant en moyenne les meilleures capacités de dispersion, c'est-à-dire avec les meilleurs taux de fructifications et de germination de basidiospores. Ces génotypes ne correspondraient pas nécessairement aux phénotypes plus virulents. Toutefois, l'association entre dispersion et virulence pour *A. ostoyae* reste à tester.

2. Parasite et/ou saprophyte

L'intensification des méthodes sylvicoles dans le massif forestier des Landes de Gascogne, en plus d'accroître la fréquence des facteurs favorisant le développement des pathogènes, conduit également à une augmentation de la disponibilité en bois morts dans les peuplements de pins maritimes par l'augmentation de souches d'arbres si elles sont laissées en place après la récolte finale (Brin *et al.* 2008). Cette intensification pourrait donc contribuer à une

évolution différente du pathogène entre les forêts naturelles et les forêts de plantations. La phase parasitaire et la phase saprophytique, qui font intervenir différents complexes enzymatiques agissant sur différents composés du bois, suggèrent l'installation d'un compromis évolutif entre ces deux stratégies évolutives. L'importante agressivité d'*A. ostoyae* constatée dans le massif forestier aurait donc pu se faire au détriment de la phase saprophyte du pathogène. Cependant, en accord avec l'étude de Prospero *et al.* (2004), l'évaluation des différentes composantes de l'agressivité et du saprophytisme ne révèle pas l'existence d'un tel compromis entre ces deux phases du cycle biologique d'*A. ostoyae*. L'absence de relation entre ces deux phases, qu'elle soit positive ou négative, suggère donc que ces deux stratégies semblent évoluer indépendamment l'une de l'autre. Un isolat d'*A. ostoyae*, qui présente une forte agressivité, peut tout aussi bien présenter une faible ou une importante capacité saprophyte. L'intensification des méthodes de gestion sylvicole dans le massif forestier ne semble donc pas avoir favorisé une stratégie au dépend d'une autre. Ces observations reposent toutefois sur des mesures de traits que l'on suppose représentatifs de ces phases du cycle biologique du pathogène. L'identification des gènes impliqués dans la virulence et le saprophytisme, couplée à des analyses de transcriptome du pathogène, permettrait de tester, à une échelle plus fine, cette absence de compromis.

III. Reconstruction de l'histoire démographique d'*A. ostoyae*

Comme présentée en introduction, l'histoire démographique récente de l'Armillaire dans le massif forestier des Landes de Gascogne ne reposait, jusqu'à aujourd'hui, que sur des hypothèses basées sur des signalements de la maladie apparemment en nombre croissant sur le massif (Lévy & Lung-Escarmant 1998; Aumonier 2007). Les deux approches choisies, épidémiologique et génétique, ont permis non seulement de préciser l'origine probable de l'expansion mais d'apporter des éléments nouveaux sur l'histoire démographique d'*A. ostoyae* sur le massif.

1. L'ancien déclin

L'approche ABC employée dans mon étude indique que la population landaise d'*A. ostoyae* aurait subi une forte réduction ancienne de sa taille efficace de l'ordre de 90% environ il y a environ 1700 générations avant notre ère. Cet épisode démographique paraît cohérent avec l'important changement démographique des forêts lors du dernier épisode glaciaire. *A. ostoyae* pouvant parasiter beaucoup d'espèces différentes de conifères de

l'hémisphère nord et pouvant également coloniser tout type de bois mort, l'hypothèse d'une histoire démographique commune entre *A. ostoyae* et le pin maritime peut sembler forte. Toutefois, l'histoire démographique du pin maritime est très représentative de l'évolution de la couverture de la forêt dans les Landes de Gascogne, et donc des autres hôtes potentiels de l'agent pathogène. Par exemple, lors de la dernière glaciation, à l'exception de quelques îlots de forêts boréales dans les zones les plus au sud de l'Europe de l'ouest, les espèces ligneuses sont donc pour la plupart absentes de la région qui est majoritairement recouverte par la toundra (Frenzel *et al.* 1992). De plus, bien qu'*A. ostoyae* puisse persister plusieurs dizaines années dans des souches par exemple, celui-ci ne peut cependant pas se maintenir des centaines, voire des milliers d'années dans des sols dépourvus d'arbres, comme cela fut le cas pendant la dernière glaciation. Il est donc probable que quelques populations relictuelles d'*A. ostoyae* aient pu survivre dans des îlots refuges de la région, et participer à la recolonisation. Une autre hypothèse serait une recolonisation avec un fort effet fondateur à partir d'une population plus au sud, en Espagne par exemple, où il restait toujours du pin maritime. Des études de phylogéographie comparant ces populations seraient intéressantes à mener pour confirmer l'une ou l'autre hypothèse.

2. La récente émergence

Des gradients de colonisations ont déjà été identifiés chez de nombreux pathogènes de plantes et d'animaux (Barrès *et al.* 2008; Robert *et al.* 2012; Fontaine *et al.* 2013). Mais il s'agit en général de colonisation sur de larges surfaces à l'échelle de continents, voir entre continents. Mon travail abordait une échelle spatiale plus réduite et a permis malgré tout d'identifier de telles signatures d'expansion. En effet, il peut apparaître remarquable que la distribution spatiale actuelle de la maladie témoigne encore aujourd'hui de la distribution ancienne du pathogène, il y a plus de 150 ans. Ceci est probablement associé à ses faibles capacités de colonisation discuté dans le chapitre IV, ainsi que par son aptitude à persister dans le sol en tant que saprophyte pendant plusieurs années (Rishbeth 1972). Les nouveaux foyers s'installent ainsi durablement, mais en général à proximité d'anciens foyers comme ce que suggère la structure génétique d'isolement identifiée à l'échelle du kilomètre dans le chapitre IV, et le lien entre forêts préexistantes et nombre de foyers à l'intérieur et à proximité identifié au chapitre II. D'autre part, cette progression à partir des anciens foyers est probablement lente sur le massif, du fait d'une probabilité d'installation par les spores aériennes et de persistance de la plupart des nouveaux génotypes installés récemment qui pourraient être faibles. De tels gradients de colonisation récents et limités à quelques hectares seraient donc à rechercher chez des organismes ayant des caractéristiques biologiques

similaires à *A. ostoyae*, puisqu'il est prévisible que pour ceux à dispersion plus efficace, le signal spatial d'expansion s'effacerait vite à cette échelle. Néanmoins, l'importance du type de marqueurs moléculaires utilisés pour ce type d'étude est aussi à considérer. La vitesse d'apparition des mutations peut permettre parfois de retracer ce type d'expansion récente à l'échelle d'une région comme en témoignent les études sur les virus (par exemple Biek *et al.* 2007). On pourrait attendre un signal comparable avec *Heterobasidion annosum*, mais sa distribution plus à l'est (Aumonier 2007) reste une énigme qu'une approche identique à celle abordée dans ce travail pourrait peut-être clarifier.

La répartition géographique actuelle d'*A. ostoyae* suggère ainsi que le pathogène aurait été présent initialement dans plusieurs fragments forestiers que comptaient la région avant que les plantations de pin maritime de la seconde moitié du 19^{e} siècle n'en recouvrent la quasi-totalité. Le changement paysager, par l'homogénéisation du paysage et une intensification des connexions entre les sources du pathogène, aurait contribué à accroître l'aire de répartition d'*A. ostoyae* dans la région comme il est souvent supposé pour d'autres pathogènes (Delatour *et al.* 1985; Perkins & Matlack 2002; Jules *et al.* 2002; Stukenbrock & McDonald 2008). Contrairement aux populations natives, les populations récemment introduites subissent le plus souvent une succession d'événements de goulots d'étranglement, qui s'accompagne d'une diminution de la diversité génétique (Prugnolle *et al.* 2005). Cette réduction du polymorphisme dans les zones colonisées par le pathogène était donc attendue dans cette étude. Toutefois, l'absence de différenciation génétique significative constatée entre les individus issus des forêts préexistantes ne permet pas de déterminer précisément la source de la maladie. La difficulté d'identifier les aires natives d'*A. ostoyae* par des approches moléculaires pourrait s'expliquer par la multitude des forêts préexistantes qui ne sont pas génétiquement ou faiblement différenciées. Ainsi, ce grand nombre de sources potentielles du pathogène couplé aux cas de dispersion à longue distance du pathogène, suffirait à homogénéiser génétiquement la population d'Armillaire à l'échelle d'étude considérée ici.

Cette émergence de l'Armillaire dans le massif forestier des Landes Gascogne est de plus partiellement soutenue par l'approche ABC. Bien que cela ne soit pas le scénario montrant la plus forte probabilité, une expansion récente il y environ 5 générations, compatible avec le développement de la sylviculture dans les Landes il y a une centaine d'années, a été identifié avec une probabilité proche du premier scenario. Plusieurs facteurs peuvent avoir contribué au fait que le scénario d'expansion ne soit pas identifiée comme le plus probable (comme expliqué dans le chapitre IV), notamment le long temps de génération du pathogène en relation avec le fait que cet événement se soit produit très récemment produisant un faible

signal génétique peu discernable des événements antérieurs (voir Girod *et al.* 2011). La mise en place d'un suivi temporel de la maladie permettrait de mieux confirmer cette expansion. Cependant, un tel dispositif nécessite d'importants moyens humains et financiers afin d'identifier l'apparition des nouveaux foyers de la maladie ainsi que de suivre l'évolution des anciens foyers à la fois dans l'espace et dans le temps. Le développement d'une grande quantité de nouveaux marqueurs moléculaires neutres chez *A. ostoyae*, est une autre alternative qui permettrait aux approches ABC de gagner en puissance, et donc, de potentiellement mettre significativement en évidence ce phénomène récent.

IV. Vers une meilleure gestion des risques liés à *A. ostoyae* dans les Landes

Les résultats de ma thèse confirment que même si la maladie s'installe lentement dans le massif forestier, celle-ci semble progressivement envahir et provoquer de plus en plus de dégâts dans le massif, que ce soit en raison de l'accroissement du nombre de sites infectés, ou en raison d'une possible amplification de la virulence de l'agent pathogène, qui reste pour ce dernier point à démontrer. Cette augmentation de virulence dans les prochaines années est cependant difficile à prédire car l'évolution d'*A. ostoyae* peut fortement dépendre des méthodes sylvicoles pratiquées, mais aussi des interactions entre les différents traits biologiques de l'agent pathogène qui peuvent être complexes. Actuellement, les sylviculteurs se préoccupent peu des dégâts provoqués par l'Armillaire obscure, car, à l'exception des plantations de pins à proximité de zones forestières préexistantes ainsi que le long du littoral atlantique, les mortalités se manifestent rarement de manière spectaculaire. De plus, en raison des lésions latentes racinaires, seule une partie des arbres infectés par l'Armillaire est visible actuellement. Cependant, en l'absence de méthode de lutte efficace et considérant l'expansion spatiale fortement suggérée par mes travaux, il est probable que le plateau landais, qui ne diffère pas fortement ni climatiquement, ni pédologiquement de la zone côtière, puisse à l'avenir subir autant de dégâts que les forêts dunaires. Une des questions en suspend est notamment de prévoir l'évolution des nombreuses mortalités associé à quelques arbres qui ont été identifiées lors de mes prospections. A cela il faut ajouter vraisemblablement de très nombreuses infections invisibles sur le massif car, en raison des lésions latentes racinaires supposées se développer chez de nombreux pins de stade intermédiaire, seul une partie des arbres infectés par l'Armillaire est visible actuellement ce qui accentue les potentialités d'augmentation rapide des mortalités sur le massif dans les prochaines années. Il est aussi attendu que des foyers initialement limités à des arbres isolés puissent évoluer vers de grands ronds de mortalité de plusieurs hectares par dispersion progressive d'arbres en arbres au cours

des prochaines révolutions forestières. La question de la vitesse d'augmentation de ces dégâts reste cependant entière à l'issue de cette thèse car nous ne disposons pas actuellement de la dynamique temporelle des ronds de mortalité et de l'installation des nouveaux foyers par les basidiospores sauf pour quelques parcelles (Lung-Escarmant & Guyon 2004). Enfin, une autre piste à étudier plus soigneusement est le rôle des feuillus dans la forêt landaise (arbres de sous-étage, lisière ou rypisylve) comme potentiel réservoir d'inoculum. *A. ostoyae* est en effet parfois observé dans le substrat ligneux du sol associé à ces feuillus, ce qui pose la question de l'implication de ces essences dans le maintien et la propagation de la maladie.

Dans les peuplements aux fortes densités d'Armillaire, il pourrait être envisagé dans un premier temps de remplacer le pin maritime par des plantations de feuillus ou d'autres espèces de pins plus résistants à l'Armillaire, comme le pin de Monterey (*Pinus radiata*) ou même le pin noir de corse (*Pinus nigra* subsp. *Laricio*) (Lung-Escarmant & Taris 1988). Toutefois, le pin maritime correspond à l'une des espèces forestières les mieux adaptées à la fois aux sols et au climat de la région et répond à la demande croissante du bois d'industrie, notamment pour l'utilisation en tant que bois-énergie. Il est par conséquent difficilement envisageable pour les sylviculteurs landais de substituer le pin maritime pour la plus grande partie de la production actuelle. En revanche, il pourrait être souhaitable, dans les peuplements touchés par les pourridiés, d'alterner les rotations de pins maritimes d'origine landaise avec d'autres origines du pin maritime (Maroc et Corse par exemple). En effet, Lung-Escarmant & Taris (1988) ont démontré qu'elles présentaient une meilleure tolérance au pathogène en conditions naturelles. La meilleure résistance de ces provenances étant souvent associée à de plus faible croissance, il est par conséquent nécessaire, bien que difficile, d'adapter le choix de la provenance en fonction de la pression d'inoculum d'*A. ostoyae*. En revanche, l'utilisation de différentes provenances landaises de pin maritime, pour laquelle cette étude ne montre pas de différence de sensibilité à l'Armillaire ne semble pas conseillée. Cependant, tester la sensibilité des provenances des pins maritimes n'était pas l'objectif principal des inoculations artificielles utilisées. En conséquence, ces expériences ne présentent pas un nombre suffisant de répétitions pour conclure objectivement à l'absence de différence de sensibilité des diverses provenances landaises de pin maritime utilisées. De plus, ces comparaisons de sensibilités des provenances de pin maritime ont uniquement été testées en serre et, par conséquent, nécessiteraient l'installation d'une expérience équivalente, mais sur des dispositifs *in situ*, afin de confirmer ce résultat.

Dans un second temps, il serait également préférable de réduire la pression d'inoculum, par le retrait des souches et des débris de bois mort, afin de diminuer les pertes économiques

engendrées par *A. ostoyae* (Cleary *et al.* 2013). En effet, l'intensité des mortalités en forêt de plantations résulte le plus souvent de l'importance de l'inoculum initial, majoritairement contenu au niveau des souches et des débris ligneux de bois de la précédente plantation (Legrand *et al.* 2005). De plus, ces substrats peuvent alors potentiellement servir de nouveau lieu de germination des basidiospores et donc être à l'origine du développement d'un nouveau foyer de la maladie (Rishbeth 1978, 1988; Hood *et al.* 2008). L'efficacité de cette méthode a déjà été prouvée aussi bien lors des coupes à blanc que lors des éclaircies (Morrison & Mallett 1996), et repose alors sur l'élimination de la plus grande quantité de fragments ligneux tout en limitant leur dispersion (Morrison *et al.* 1988, 2014; Roth *et al.* 2000; Omdal *et al.* 2001). Enfin, rallonger la durée espaçant deux plantations et réduire la durée des rotations des peuplements, permettraient, si elles sont couplées au retrait des souches, d'épuiser et de réduire l'inoculum du sol. En effet, des parcelles de pins trop âgés et fortement impactés par l'Armillaire, contribuent significativement à l'intensification de la quantité d'inoculum dans le sol, ce qui pourrait donc maintenir le parasite dans le sol même après la coupe rase et entraîner d'importants dégâts dans la future nouvelle plantation. Inventer une sylviculture moderne faite de mélanges et de nouvelles méthodes de gestion est un défi. Toutefois, de plus en plus de risques sont aujourd'hui pris en compte et, comme en témoigne cette thèse, les risques associés à l'Armillaire devront y être rapidement intégrés.

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Titre : Étude de l'émergence et de la dynamique évolutive d'*Armillaria ostoyae*, agent pathogène du pin maritime

Résumé : Dans la forêt de pin maritime (*Pinus pinaster*) des Landes de Gascogne (sud-ouest de France), la mortalité des pins causée par le champignon pourridié *Armillaria ostoyae* (Basidiomycète) a augmenté au cours des 30 dernières années. Les premiers cas de cette maladie ont été signalés quelques années après un changement majeur dans l'utilisation des terres, qui a eu lieu dans cette région suite au remplacement des landes et marais d'origine par une forêt plantée et gérée da façon intensive. Notre objectif était de comprendre les facteurs à l'origine de cette maladie émergente. Pour cela, nous avons étudié la distribution spatiale des dommages causés par le pathogène en relation avec des facteurs historiques, estimé la variabilité des traits fongiques liés au parasitisme et saprophytisme, et étudié l'histoire démographique d'*A. ostoyae*. La répartition actuelle de la mortalité induite par *A. ostoyae* est apparue dépendre de la présence des forêts préexistantes, ce qui suggère qu'*A. ostoyae* était fréquent dans ces zones forestières anciennes, qui ont agi comme un réservoir pour la colonisation des forêts plantées récentes. La production de rhizomorphes était significativement corrélée avec la virulence, suggérant que ce trait joue un rôle important dans le stade parasitaire d'*A. ostoyae*. Aucune relation significative entre le parasitisme et saprophytisme n'a été détectée, suggérant une absence de compromis évolutif entre ces traits. Enfin, le meilleur scénario démographique pour expliquer la structure de la population d'*A. ostoyae* dans la forêt des Landes est un scénario en deux étapes : il y aurait eu d'abord une diminution puis une expansion de la population fongique, qui semblait suivre la dynamique de la population d'hôtes. Le temps de génération d'*A. ostoyae* a été estimé entre 10 et 20 ans.

Mots clés : Champignon pathogène des forêts, *Pinus pinaster*, agents de pourridiés, maladie émergente, changements paysagers, Analyses Bayésiennes Approximées, expansion, temps de génération, forêts de plantations

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Title: Study of the emergence and evolutionary dynamics of *Armillaria ostoyae* a pathogen of maritime pine

Abstract: In the maritime pine (*Pinus pinaster*) forest of the Landes de Gascogne (south-western France), pine mortality due to the root rot fungus *Armillaria ostoyae* (Basidiomycete) has been increasing over the last 30 years. The first cases of this disease were reported a few years after a major change in land use which occurred in this region following the replacement of original moors by an intensively managed planted forest. Our aim was to understand the factors driving this disease emergence. For this, we investigated the spatial distribution of pathogen damage related to historical factors, estimated the variation in fungal traits related to parasitism and saprophytism and investigated the demographic history of *A. ostoyae*. The current distribution of *A. ostoyae* mortality appeared depending on the pre-existing forests, suggesting that *A. ostoyae* was commonly distributed in pre-existing forest areas which acted as a reservoir for the colonization of recent planted forests. The rhizomorphs production was significantly correlated with virulence, suggesting that this trait plays an important role in the parasitic stage of *A. ostoyae*, but no significant relationship between parasitism and saprophytism components was detected, which may suggest that there is no trade-off between these traits. Finally, the best demographic scenario to explain *A. ostoyae* population structure in the Landes forest is a two step scenario: there was first a decrease and then an expansion in the fungal population, which appeared to follow the dynamics of the host population. The generation time of *A. ostoyae* was estimated between 10 and 20 years.

Keywords: Fungal forest pathogen, *Pinus pinaster*, root-rot disease, disease emergence, land-use change, evolutionary trade-off, virulence, wood degradation, Approximate Bayesian Computations, expansion, generation times, planted forests

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